UNIVERSIDADE DE LISBOA
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THE USE OF SUBCLINICAL VASCULAR MARKERS OF ATHEROSCLEROSIS IN YOUTH

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"Bloom procurava o insólito que não
sendo acontecimento mudo ou ruído, sendo
sítio, obriga a caminhar. Se o que procuro
chegasse à minha cadeira,
para que serviriam os sapatos? Mas é já
um conhecimento clássico: acontecimentos novos
existem em espaços novos, e não em antigos.
Não deixes que a tua cadeira confortável prejudique
a tua curiosidade."

Gonçalo M. Tavares
in Uma Viagem à Índia,
canto 1, estrofe 70

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This thesis is part of a discovery journey with no particular destination at sight other than to go. But what then? What have I really gained by all this trouble? What have I brought back from this long and weary journey? Nothing, some might say? Perhaps so, nothing but charming achievements, who, strange as it may appear, made me sometimes the happiest of men, even if were meaningless to others. Truly, would you not for less than that start this journey from scratch again? After all, it's not my professional or academic achievements that set my mind at ease. It is how much I have traveled.

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ABBREVIATIONS

AUC	Area under the curve
ABFI	Abdominal fat index by DXA
BMI	Body mass index
cIMT	Carotid artery intima-media thickness
CRF	Cardiorespiratory fitness (VO_2max)
CVD	Cardiovascular disease
DXA	Dual-energy X-ray absorptiometry
HR	Heart rate
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
MSI	Muscular strength index
PP	Pulse pressure
PWV	Pulse wave velocity

P75 75th percentile

ROC Receiver operating characteristic

SD Standard deviation

SED Sedentary behavior

TBF Total body fat

TBFI Total body fat index

TBLST Total body lean soft tissue

TBLSTI Total body lean soft tissue index

TF Trunk fat by DXA

WC Waist circumference

WHtR Waist-to-height ratio

ABSTRACT

The foundations for cardiovascular disease (CVD) in adults are laid in childhood and accelerated by the presence of comorbid conditions. Early detection of manifestations of cardiovascular pathology is an important clinical objective to identify those at risk for subsequent cardiovascular morbidity and events, and to initiate behavioral and medical interventions to reduce risk. Children were once considered to be at low risk, but with the growing health concerns related to lifestyle, cardiovascular screening may be needed earlier. Several noninvasive procedures are available to assess the cumulative effect of these exposures. These include carotid ultrasound, flow-mediated dilation, pulse wave velocity and measures left ventricular mass. This dissertation analyzes the comorbid conditions that increase cardiovascular risk in youth, namely obesity and low physical fitness, using carotid intima-media thickness to objectively detect early manifestations of cardiovascular pathology.

Until recently researchers have not used surrogate markers of subclinical atherosclerosis to examine the role of a single bout of exercise. Utilizing the acute exercise model can be advantageous as it allows for an efficient manipulation of exercise variables and permits greater experimental control of confounding variables. It is possible that the effects of a bout of exercise can predict the effects of chronic exercise. We analyze the physiological factors pertinent to arterial stiffness using arterial distensibility and pulse wave velocity in the context of acute exercise in children and adults.

In some instances, those who amend their trajectory by not maintaining risk factors into adulthood experience reductions in subclinical markers to levels associated with never having had the risk factor. Though avoidance of risk factors in youth is ideal, there is still a window for intervention where long-lasting cardiovascular effects might be avoided. In this dissertation we present preliminary findings linking modifiable youth risk factors to subclinical markers of CVD in adulthood.

KEYWORDS

Cardiovascular Disease; Carotid Intima-Media Thickness; Arterial Stiffness; Modifiable Risk Factors; Children And Adolescents; Acute Exercise; Cross-Sectional; Longitudinal

RESUMO

As bases da doença cardiovascular (DCV) em adultos são estabelecidas na infância e aceleradas pela presença de comorbidades. A deteção precoce de manifestações da patologia cardiovascular é um objetivo clínico importante na identificação daqueles com risco de subsequente morbidade e eventos cardiovasculares, e no estabelecimento de intervenções comportamentais e médicas para reduzir o risco. As crianças já foram considerados de baixo risco, mas com as crescentes preocupações de saúde associadas ao estilo de vida, o rastreio cardiovascular é cada vez mais precoce. Vários procedimentos não invasivos estão disponíveis para avaliar o efeito cumulativo dessas exposições. Estes incluem ultrassom da artéria carótida, a dilatação fluxo-mediada, velocidade de onda de pulso e medidas da massa ventricular esquerda. Esta dissertação analisa comorbidades conhecidas que aumentam o risco cardiovascular em crianças e adolescentes, como a obesidade, pressão arterial elevada e baixa aptidão física, usando a espessura da parede intima-media da carótida para detetar objetivamente as manifestações precoces de patologia cardiovascular.

Até recentemente, estes marcadores subclínicos de aterosclerose foram pouco utilizados para examinar os efeitos de uma única sessão de exercício físico. No entanto, a utilização do modelo de exercício agudo pode ser vantajoso, pois permite uma manipulação eficiente das variáveis do exercício e permite maior controle experimental de variáveis de enviezamento. É possível que os efeitos de uma sessão de exercício possam prever os efeitos do exercício crónico. Nesta dissertação analisamos os fatores fisiológicos associados à rigidez arterial usando a distensibilidade arterial e velocidade da onda de pulso no contexto de exercício agudo em crianças e adultos.

Em alguns casos, aqueles que melhoram o seu perfil de risco de risco para as DCV até à idade adulta experienciam reduções em marcadores subclínicos de aterosclerose para níveis saudáveis. Embora a prevenção de fatores de risco na juventude seja o ideal, existe ainda uma janela para a intervenção em que os efeitos cardiovasculares de longa duração pode ser evitada. Nesta dissertação apresentamos resultados preliminares que ligam fatores de risco modificáveis na juventude com marcadores subclínicos de DCV na idade adulta.

PALAVRAS CHAVE

Doença Cardiovascular; Espessura Da Parede Íntima-Média Da Artéria Carótida; Rigidez Arterial; Fatores De Risco Modificáveis; Crianças E Adolescentes; Exercício; Efeitos Agudos; Transversal; Longitudinal.

PRELUDE

SURROGATE VASCULAR MARKERS

1. INTRODUCTION

Global mortality due cardiovascular diseases (CVD) is currently the world's leading cause of death and is projected to remain the number one cause of global mortality [2]. Most cases of this disease are preventable, and the extent and progression of the disease from childhood to adulthood are positively associated with both lipid and non-lipid risk factors.

Over recent decades the interest in cardiovascular epidemiology has broadened from studies on causes and consequences of elevated cardiovascular risk factors to include research on causes and consequences of atherosclerosis and associated arterial wall abnormalities. One of the underlying reasons was that established cardiovascular risk factors were insufficiently accurate in identifying those individuals who will suffer from CVD in the future and measures of subclinical atherosclerosis may enhance the precision of these predictions and thus enable better-tailored medical care to be provided [3].

Studies have reported that risk factor profile assessed in adolescents predicts adult common carotid artery intima-media thickness (cIMT) independently of contemporaneous risk factors [4], suggesting that exposure to cardiovascular risk factors early in life may induce changes in arteries that contribute to the development of atherosclerosis and that exposures to risk factors at a young age leave a lasting imprint. The increase in the lifetime risk of developing coronary heart disease irrefutably attest to the importance of diagnosis early in the natural history of CVD and appropriately matching the intensity and type of intervention with the extent of disease and tempo of disease progression [5].

The atherogenic effects of various risk factors are not necessarily equivalent in children and adults. In boys for example, smoking - the primary risk factor for CVD in adults - was associated with significantly less atherosclerotic lesion formation than obesity in a multi-institutional autopsy study conducted in US medical centers [6]. This is possibly because smoking is a thrombotic risk factor and therefore has more effects in the progression of, rather than formation of early atherosclerotic lesions [6]. Studies in older adults can be clouded by the effects of environmental and genetic factors, not all of which may be identifiable. Thus, studies on the pathogenesis and treatment of CVD beginning in childhood and young adulthood may provide a better understanding of this disease.

Because of inherent physiological differences in children, the diagnosis and treatment of youth at risk for CVD cannot be simply extrapolated from adult data [5].

2. SURROGATE VASCULAR MARKERS

In the adult population, it has become increasingly common to utilize intermediate endpoints, such as cIMT or arterial stiffness, rather than a clinical endpoint such as myocardial infarction [5]. Results obtained with the commonly used surrogate endpoints should be cautiously considered, and the assessment of treatments should, when possible, be based on clinical rather than intermediate endpoints but the substitution has been on the way for both practical and financial reasons. An intermediate endpoint is defined by Boissel et al. [7] as a response variable that is statistically correlated with the clinical endpoint. In some instances, an intermediate endpoint is a surrogate endpoint, defined as a response variable that is an alternative to a clinical endpoint. The use of a surrogate endpoint in medicine is common, e.g. changes in blood pressure, hemoglobin A_{1C} and cholesterol levels are used to assess the effects of therapeutic interventions intended to prevent the pathogenic cardiovascular sequelae of hypertension, diabetes and hyperlipidemia. Multiple clinical trials have clearly proven the association of these variables with CVD endpoints [5].

The progression from the earliest arterial changes associated with atherosclerosis and the clinical manifestations of CVD commonly occurs over many decades except in rare cases such as in children with homozygous familial hypercholesterolemia where it is greatly accelerated. Thus, studies linking these variables with CVD endpoints in youth are generally lacking and treatment algorithms for hypercholesterolemia and hypertension in youth for example, are based on population-derived endpoints [5]. The use of surrogate endpoints, such as cIMT or arterial stiffness, provides an alternative approach that may better identify youth with or at risk for CVD, especially those with multiple CVD risk factors. A valid surrogate marker of CVD in the pediatric population should be capable of predicting the risk for developing CVD as an adult. Ideally, implementation of disease-modifying therapies should cause improvement or normalization of such markers [8].

Low-density lipoprotein cholesterol is commonly used as a surrogate marker of CVD risk, but may be unsatisfactory in children as cholesterol levels have a polyphasic pattern of

change with age and do not track as well as weight and height from childhood to adulthood [9]. Plus, the effects of additional cardiovascular risk factors, which are increasingly prevalent, may act synergistically to accelerate the progression of CVD. The use of population-based norms for discrete cardiovascular risk factors may significantly underestimate the additive effects of multiple risk factors [5]. It is therefore imperative that researchers distinguish and utilize markers of early arterial injury to better identify high-risk youth and to develop effective treatment strategies for the pediatric population.

The scientific knowledge of the pathogenesis of atherosclerosis has progressed to the molecular understanding of the aggression to the endothelium - the first defense line against CVD. With high-resolution ultrasound techniques to assess vascular function, researchers can now surpass anatomical and functional evidence for atherogenesis from invasive investigations or postmortem examination [10]. The most widely used test to evaluate endothelial function measures the vasodilator response to increased blood flow of the brachial artery (flow-mediated vasodilatation) [11]. Studies in adults have demonstrated a close association between the endothelial function of the peripheral circulation and that of the coronary circulation. The remodeling of the large arteries can be evaluated by measuring the thickness of the intima and media layers of the common carotid arteries, or of the aorta [12]. These approaches provide an estimation of the examined arterial bed. However, a more global evaluation of the physical properties of the arterial system can be given by measuring the pulse wave velocity that derives from complex interactions between the ventricular performance, the physical properties of the arterial system, and rheological characteristics of the blood [13]. Additionally, analysis of the pulse waveform by applanation tonometry for example, provides a noninvasive means to record central aortic pressure and waveform conveying important information about cardiovascular status [14].

The surrogate vascular markers described in this dissertation are examples of the earliest, measurable toxic effects of risk factors on arterial health and are increasingly being used in pediatric clinical trials. Each is more tightly linked with the cause-effect relationship between hyperglycemia, dyslipidemia, hypertension, obesity and smoking and the molecular underpinnings of atherosclerosis than the isolated risk factor values [5]. Surrogate vascular markers provide the means for objectively evaluating the benefits that are expected to derive from lifestyle intervention in this population.

3. THE AIMS OF THE DISSERTATION

This dissertation describes our scientific contribution on the use of noninvasive techniques for assessing vascular health in youth, bringing new data to think normal patterns of development and aging. In addition we report evidence that cardiovascular risk factors in childhood are associated with vascular pathology. We review the likelihood of reversibility of pathology following exercise interventions in youth and examine the role of a single bout of exercise on the arterial tree, as it is possible that the effects of a bout of exercise can predict the effects of chronic exercise. Because long-term clinical trials to test the potential benefit of exposure modification in youth on development of CVD in adulthood are not possible, we present preliminary data from a cohort study with measurements across the life-course providing the best opportunity to understand the potential effects of risk factors on an individual's path toward CVD.

4. DISSERTATION STRUCTURE

The present dissertation incorporates a compilation of 6 research articles already published, in press, or submitted for publication in peer-review journals with an established ISI Impact Factor, and 1 manuscript from preliminary data of an ongoing project. To clarify the framework of these studies this dissertation is organized in the following way:

CHAPTER 1: CAROTID INTIMA-MEDIA THICKNESS

Briefly summarizes our starting point on the results of cIMT in children and adds our own scientific contribution on the association of obesity phenotypes and fitness with cIMT, including speculation regarding underlying mechanisms leading to overt CVD that occurs typically later in adulthood, and the likelihood of reversibility of pathology following interventions. A succinct reference to the methods of assessment of cIMT is provided in the background and later complemented in detail in the articles. Specific updated findings on the cIMT and insightful discussion topics with peers may be found in the articles.

CHAPTER 2: ARTERIAL STIFFNESS

Briefly summarizes our starting point on the results of arterial stiffness in children with evidence that cardiovascular risk factors are associated with arterial stiffness, including speculation regarding underlying mechanisms leading to overt CVD that occurs typically later in adulthood, and the likelihood of reversibility of pathology following interventions. In addition we summarize our starting point on the expanding application of arterial stiffness in the acute exercise paradigm and add our own scientific contribution, discussing the physiological factors pertinent to arterial stiffness in the context of acute exercise in children and adults. A succinct reference to the methods of assessment of arterial stiffness is provided in the background and later complemented in detail in the article. Specific updated findings on arterial stiffness may be found in the article.

CHAPTER 3: WHEN TO PREVENT CARDIOVASCULAR DISEASE

Briefly summarizes recent developments and adds our own scientific contribution examining whether the trajectories, from adolescence to young adulthood, of blood pressure, body fatness and fat distribution, cardiorespiratory fitness (CRF) and physical activity, determined levels of arterial structure and stiffness in young adults. We make use of this useful conceptual model to understand the importance of time and timing in associations between CVD risk factors and vascular health.

CHAPTER 4: WHERE ARE WE AND WHERE ARE WE GOING?

Discusses the main findings from this dissertation, their clinical implication and remaining gaps in knowledge. Focus is then directed to imaging modalities and autonomic function that are very promising tools to expand the understanding of the Exercise and Health Laboratory of the Faculty of Human Kinetics on the impact of CVD on youths.

CHAPTER 1

CAROTID INTIMA-MEDIA THICKNESS

1. BACKGROUND

Assessment of carotid intimal and medial thickness (cIMT) with high-resolution B-mode ultrasonography has emerged as one of the more powerful tools for the evaluation of subclinical atherosclerosis over the last 2 decades. Newer ultrasound systems with high-frequency transducers allow easy identification of the lumen-intima interface and intima-adventitia interface and thus easy and reliable measurement of cIMT. Far-wall cIMT accurately represents the intima-media thickness compared with direct histological examination [3].

RELATIONSHIP TO CARDIOVASCULAR RISK IN ADULTS

In adults, increased cIMT is associated with coronary artery disease and is predictive of future cardiovascular events, including stroke and myocardial infarction [15-20]. Several cardiovascular risk factors have been associated with cIMT, including age, male sex, diabetes mellitus, total cholesterol, and smoking [21-23]. However, the added value of measurement of mean cIMT on the 10-year risk prediction of first-time myocardial infarction or stroke has only been found to be significant in individuals at intermediate risk [24].

cIMT has proved sufficiently robust and reproducible in the evaluation of changes over time to serve as an end point in clinical trials assessing the impact of antihypertensive and lipid-lowering medications on cardiovascular risk [25-30] but the association between cIMT progression and cardiovascular risk is more frequently assumed than it has been proven [31]. An extensive review of randomized controlled trials evaluating the effect of an intervention on cIMT is available [32].

RELATIONSHIP TO CARDIOVASCULAR RISK IN CHILDREN AND ADOLESCENTS

Increased cIMT and various elevated cardiovascular risk factors in young obese subjects have been shown [33, 34]. cIMT has been found to be 9–25% higher and directly correlated to body mass index (BMI) in obese children, independent of vessel size [35]. However, not all studies have found increased cIMT in obese children [36].

Metabolic syndrome in children appears to further deteriorate arterial structure. Augmented wall thickness and increased lumen diameters, coupled with a greater cross-sectional area of the intima-media complex compared with healthy control children were found in obese children with metabolic syndrome [37]. However, even if arterial structural changes were detectable at this young age in presence of metabolic syndrome, this study did not compare the children with metabolic syndrome to obese children without metabolic syndrome, thus the unique effects of metabolic syndrome could not be determined.

Increased cIMT is a common finding among children with familial hypercholesterolemia [38, 39]. The exact mechanisms for these changes in arterial structure remain unclear but are probably related to an excessive, inflammatory-fibroproliferative response to the insults of hypercholesterolemia to the endothelium and smooth muscle of the artery wall with the participations of a large number of growth factors, cytokines and vasoregulatory molecules [40]. However, serum cholesterol levels have not always been associated with decreased arterial structure [41], suggesting a more complex interactive process.

Children with primary hypertension are frequently overweight leading to difficulty in discerning the possible effects of hypertension on the vasculature from the strong association between obesity and increased cIMT. However, hypertensive subjects had increased cIMT compared with matched controls and higher cIMT correlated with more severe hypertension by ambulatory blood pressure monitoring providing strong evidence that cIMT is increased in childhood primary hypertension, independent of the effects of obesity [42, 43].

Several studies have shown an increase in cIMT among insulin-dependent diabetes mellitus children [44-46]. This increase in cIMT has been reported in children receiving higher insulin doses, denouncing a detrimental influence on the arterial wall. However, data in adults showed that progression of cIMT was reduced in subjects receiving intensive diabetes treatment [47]. Therefore, it is likely that a more complex relationship exists between insulin and cIMT.

Assessment of cIMT has also been used to evaluate cardiovascular risk in populations of children with other chronic medical conditions, including end-stage renal disease, systemic lupus erythematosus [48], HIV infection [49], and Kawasaki disease [50], and in patients who have undergone repair of aortic coarctation [51].

DEVELOPMENTAL ASPECTS OF ARTERIAL STRUCTURE

The developmental changes of cIMT during the developmental years have not been clearly elucidated. Some studies assessing the arterial wall dimensions and properties in a large population of healthy adolescents revealed significant variations with age and body size and markedly skewed distributions, which were in part related to concordant variations of body mass and blood pressure [52], but this finding has not been consistent, as others have found little change in cIMT during childhood [53]. Median cIMT increased linearly from 0.384 to 0.397 mm [52] and from 0.377 to 0.407 [1] between 10 and 18 years of age. Thus, if cIMT changes throughout childhood, these changes are very small and their clinical or functional relevance questionable [35]. In addition, these changes in cIMT are accompanied by increases in arterial size, including luminal diameter [52] suggesting that the increase in cIMT may be a function of increased overall arterial size.

Postmortem studies characterizing the normal histological growth of the aorta in infants and children have shown that both intimal and medial thickness and density increase from birth throughout childhood [54].

EFFECTS OF EXERCISE INTERVENTIONS

The effect of exercise training on subclinical atherosclerosis was examined in endurance trained and sedentary healthy men and found no significant difference in cIMT and cIMT/lumen ratio between groups [55-57]. However, there are discrepancies in findings [58], that may be due to differences in training intensity and/or load [59].

Longitudinal studies evaluating the potential of 8-12 weeks of exercise training to affect arterial structure found no evidence of changes in the conduit arteries in the non-trained areas [55, 60, 61]. In a subgroup analysis of a study by Rauramaa et al. [62] determining whether progressive aerobic exercise compared with usual activity slows progression of atherosclerosis in men not taking statins showed that the 6-year progression of cIMT, adjusted for smoking and annual measures of low-density lipoprotein cholesterol level, SBP, and WC, was 40% less in the exercise group than in the control group, suggesting that the anti-atherosclerotic effects of statins may mask the impact of exercise.

Although analysis of cIMT has been used extensively in cross-sectional studies in children, few clinical trials have used cIMT as an end-point. Short-term interventions appear to have little effect on vascular remodeling, suggesting functional changes precede structural changes [35]. Twelve-month long interventions have demonstrated improvements in cIMT of overweight or obese youth [63, 64] but possibly not independent from substantial weight loss [64]. It does appear that long-term lifestyle modifications hold promise in reversing early atherosclerotic changes in this population.

POSSIBLE UNDERLYING MECHANISMS

Although the physiological mechanisms responsible for the favorable outcomes in children with CVD risk factors remain speculative, they are likely multiple given the multidimensional nature of interventions [35]. In studies with adults, the relationship between physical activity and cIMT is independent of cardiovascular risk factors [65, 66]. This suggests that the effect of exercise training on arterial wall thickness cannot be entirely explained by exercise-mediated changes in traditional cardiovascular risk factors, such as lipid levels, fatness and blood pressure. Alternative pathways to explain the change in cIMT after exercise training may rely on the role of local haemodynamic stimuli (shear stress and arterial pressure) and systemic non-haemodynamic stimuli (vascular tone, sympathetic nervous system, oxidative stress and inflammation) [59]. Shear stress plays an important role in the regulation of large artery remodelling [67], and the development of carotid atheromatous plaques has been linked with the presence of low mean shear rate [68]. Studies suggest that systemic, rather than localized, shear stress plays an important role in adaptations of the arterial wall in response to exercise training [58, 69]. Chronic increases in blood pressure result in pro-atherogenic endothelial cell phenotypes, which are characterized by lower endothelial nitric oxide synthase mRNA expression and higher levels of VCAM-1, ICAM-1, ET-1 and reactive oxygen species [70]. It is speculated that up-regulation of pro- (e.g. VCAM-1, ICAM-1, ET-1 and ROS) and anti-atherogenic (e.g. endothelial nitric oxide synthase) genes differ when the pressure stimulus involves chronic elevation compared with the transient, episodic and cyclical increases in blood pressure which occur in response to exercise [70, 71]. Sympathetic nervous system activity and femoral artery IMT in healthy young and older men are strongly correlated [72]. This suggests that sympathetic nervous system activity levels may

contribute to changes in arterial wall thickness. It is currently unknown whether changes in sympathetic nervous system activity and IMT as a result of exercise training are correlated, and whether this potential relation differs between groups. Oxidative stress represents a balance between the production of reactive oxygen species and the efficiency of antioxidant defenses and is believed to contribute to the development of atherosclerosis [73, 74]. Exercise training, especially when performed at moderate-intensity levels, is associated with antioxidant effects [75]. The importance of inflammatory processes during the development of carotid atherosclerosis is commonly accepted [76]. Exercise training is associated with anti-inflammatory effects, which are believed to contribute to the cardioprotective effects of an active lifestyle [77].

2. PURPOSE

Several reviews suggest that the foundations for vascular disease in adults are laid in childhood and accelerated by the co-existence of disease [5, 35, 78, 79]. Current understanding of the effects of obesity and type 2 diabetes on vascular health in youth is incomplete. The development of noninvasive methods for assessing vascular health has made it possible to make precise and reproducible measures of vascular function and anatomic pathology early in the course of development of vascular disease, and is expected to result in the proliferation of data related to its origins.

It is possible that fitness may be at least as important, in terms of cardiovascular risk, as overweight or obesity and that the latter may possibly owe their predictive capacity to the fact that they are surrogate markers for inactivity or low fitness [80, 81]. However, limited attempts have been made to examine the complex relationships between fitness, physical activity, body composition and cIMT in children [82-85].

The purpose of this chapter of the dissertation was to gain insight into the relationships between cIMT and measures of body composition, physical fitness dimensions and physical activity in children. The research carried out on cIMT as part of the present doctoral research program resulted in the following publications, and communications (oral/poster) as first author:

PEER-REVIEWED ARTICLES THAT ARE RELATED TO cIMT

Melo, X., Santa-Clara, MH., Pimenta, NM., Carrolo, M., Martins, SS., Minderico, CS., Fernhall, B., Sardinha, LB. *Body Composition Phenotypes And Carotid Intima-Media Thickness In 11–13-Year-Old Children*. European Journal of Pediatrics, 2013. **173**(3): p. 345-352. JCR Impact Factor: 1.983 (2013). JCR Rank: Q2 (Pediatrics).

Melo, X., Santa-Clara, MH., Santos, DA., Pimenta, NM., Pinto, R., Minderico, CS., Fernhall, B., Sardinha, LB*. Single And Combined Influences Of Body Composition Phenotypes On Carotid Intima-Media Thickness In Children*. Pediatric Obesity, 2015. Jul 22. [Epub ahead of print]. JCR Impact Factor: 2.419 (2013). JCR Rank: Q1 (Pediatrics).

Melo, X., Santa-Clara, MH., Pimenta, NM., Minderico, CS., Martins, SS., Fernhall, B., Sardinha, LB. *Intima-Media Thickness In 11-13 Years-Old Children: Variation Attributed To Sedentary Behavior, Physical Activity, Cardiorespiratory Fitness And Waist Circumference*. Journal of Physical Activity & Health, 2015. **12**(5): p. 610-617. JCR Impact Factor: 1.863 (2013). JCR Rank: Q2 (Sports Science).

Melo, X., Santa-Clara, MH., Santos, DA., Pimenta, NM., Minderico, CS., Fernhall, B., Sardinha, LB. *Linking Cardiorespiratory Fitness Classification Criteria To Subclinical Atherosclerosis In Children*. Applied Physiology, Nutrition, and Metabolism, 2015. **40**(4): p. 386-392. JCR Impact Factor: 2.225 (2013). JCR Rank: Q1 (Sports Science).

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ABSTRACTS THAT ARE RELATED TO cIMT

Melo, X., Santa-Clara, MH., Carrolo, M., Pimenta, NM., Martins, SS., Minderico, CS., Fernhall, B., Sardinha, LB. *Body Composition Phenotypes And Carotid Intima-Media Thickness In 11-13 Years Old Children*. In: 7th European Youth Heart Study Scientific Symposium, 2012. Funchal. Abstract Book Of The 7th European Youth Heart Study Scientific Symposium. 2012, p. 50-50. (Oral Communication)

Melo, X., Santa-Clara, MH., Pinto, R., Pimenta, NM., Martins, SS., Minderico, CS., Fernhall, B., Sardinha, LB. *Single And Combined Influences Of Body Composition Phenotypes On cIMT Among 11-12 Years-Old Children*. In: International Syposium Of Body Composition, 2014. Cascais. European Journal Of Clinical Nutrition, 2014 (Oral Communication)

Melo, X., Santa-Clara, MH., Pimenta, NM., Martins, SS., Minderico, CS., Fernhall, B., Sardinha, LB. *Physical Activity, Cardiorespiratory Fitness And Waist Circumference: Understanding Vascular Structure In 11-13 Years-Old Children.* In: 60th Annual Meeting And 4th World Congress On Exercise Is Medicine Of The American College Of Sports Medicine, 2013. Indianapolis, Indiana. Medicine And Science In Sports And Exercise, 2013. **45**, p. 92-92. (Poster Session)

Melo, X., Santa-Clara, MH., Pimenta, NM., Martins, SS., Minderico, CS., Fernhall, B., Sardinha, LB. *Recommended Cardiorespiratory Fitness Level For Vascular Health In 11-12 Years-old Children.* In: 61st ACSM Annual Meeting, 5th World Congress On Exercise Is Medicine And World Congress On The Role Of Inflammation In Exercise, Health And Disease, 2014. Orlando, Florida. Medicine & Science In Sports & Exercise, 2014. **46**, p. 590-593. (Thematic Poster Session)

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3. METHODS OF ASSESSMENT OF cIMT

The individual layers of the carotid artery wall can be distinguished by 2-dimensional ultrasound in several locations, given the relatively superficial location and limited movement of the vessel. Studies evaluating cIMT in both children and adults have variably used a number of differing methods of analysis, including measurement of common carotid artery, carotid bulb, internal carotid artery, or an index using multiple sites, near-wall versus far-wall assessment, and differences in timing of measurements during the cardiac cycle. A measure of the combined thickness of the intima and media, or intima-media complex, is the end-point applied across all imaging protocols [79].

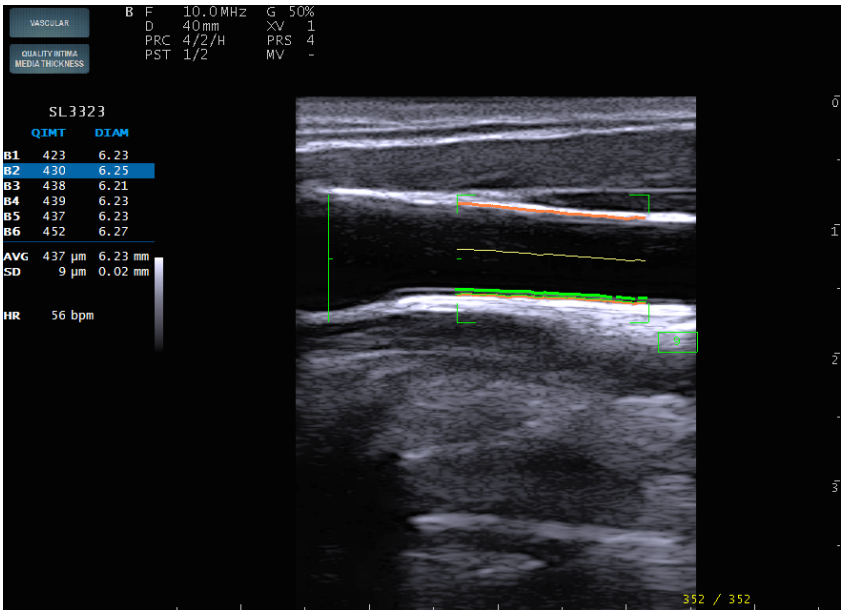

FIGURE 1: IMAGE TAKEN FROM THE COMMON CAROTID ARTERY DEMONSTRATING THE INTIMAL-MEDIAL COMPLEX AND DIAMETER OF THE CAROTID ARTERY.

Real time feedback regarding the correct probe position given during the measurement is achieved with the help of the Real Time IMT detection by displaying the following items: cIMT (µm), Diameter (µm), the last 6 beats, cIMT average and diameter average and HR in beats per minute. Standard deviation of the cIMT should be below 20 µm, and below 0.2 mm for the diameter.

Differences in the relationship between specific carotid artery sites and cardiovascular risk have been demonstrated [86]. Some studies have shown the value of common carotid artery measurements in predicting coronary events [16] and in identifying high-risk patients [19], whereas others have demonstrated that although the aggregate mean of 12 carotid

artery sites is probably the most informative in terms of describing overall atherosclerotic burden, the addition of internal cIMT assessment to analysis of the common carotid artery and carotid bulb yields little additional predictive power [87].

In this dissertation, the carotid artery was imaged with an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy) using previously validated radiofrequency-based tracking of the arterial wall (FIGURE 1) that allows a real-time determination of common carotid far-wall thickness (^{RF}QIMT) with high spatial and temporal resolution [88]. ^{RF}QIMT method is a top generation cIMT real-time measurement for high accuracy and reproducibility in early detection of CVD and for detecting early atherosclerosis.

Based on the direct analysis of the radiofrequency signal, this technique is a gold standard for diameter, changes in diameter and wall vessel measurements. The normal carotid arterial wall is composed of two acoustic impedance interfaces: the transition between blood and intima, and the transition between media and adventitia. The distance between those two acoustic interfaces is the definition for cIMT.

A detailed methodological description of this technique may be found in the following articles.

4. BODY COMPOSITION PHENOTYPES AND CAROTID INTIMA-MEDIA THICKNESS IN 11–13-YEAR-OLD CHILDREN

Melo, X., Santa-Clara, MH., Pimenta, NM., Carrolo, M., Martins, SS., Minderico, CS., Fernhall, B., Sardinha, LB. European Journal of Pediatrics, 2013. **173**(3): p. 345-352. JCR Impact Factor: 1.983 (2013). JCR Rank: Q2 (Pediatrics).

ABSTRACT

BACKGROUND: Early detection of impairment in vascular structure is an important clinical pursuit. However, it is unknown which measure of body composition best predicts vascular wall changes.

PURPOSE: We assessed the differences in body composition among carotid intima-media thickness (cIMT) tertiles and determined which measures of body composition are associated with cIMT in 385 children aged 11-13 years (196 Girls).

METHODS: In this cross-sectional study, body mass index (BMI), waist circumference (WC), total body fat (TBF) and trunk fat (TF) from DXA and cIMT through high-resolution ultrasonography were collected. Differences in body composition measures among cIMT tertiles [Low cIMT (LIMT): ≤0.46 mm; Middle cIMT (MIMT): 0.46-0.53 mm; High cIMT (HIMT): ≥0.53 mm] were assessed with ANOVA/ANCOVA after categorization. Regression analysis was used to assess the relationships between body composition and cIMT.

RESULTS: The groups were similar for sex, age and maturity ($p>0.05$). As compared with LIMT group, subjects with HIMT had higher mean values of BMI, TBF, TF, and WC ($p<0.05$). Significant differences were found for WC even when controlling for BMI ($p<0.05$). Combining all subjects, cIMT was significantly correlated to BMI, TBF, TF and WC ($p<0.05$). In multiple regression WC was the only predictor of cIMT ($\beta=0.22$, $p<0.001$).

CONCLUSION: Differences exist in body composition variables among cIMT tertiles. In the overall model WC was the only obesity related predictor of increased cIMT in 11-13 years-old children.

KEYWORDS

Children; Body composition phenotypes; Waist circumference; Intima-media thickness; Carotid artery

INTRODUCTION

The intima media thickness of the common carotid artery (cIMT) is a noninvasive, feasible, reliable and inexpensive method for detecting development of subclinical atherosclerosis [4]. The cIMT is related to the severity and extent of coronary artery disease and stroke in adults [89]. Increased cIMT has been demonstrated in pediatric patients with hypertension [42, 90], familial hypercholesterolemia [39, 91], type 1 diabetes mellitus [44, 92], obesity [93, 94], and the metabolic syndrome [95, 96].

In a recent review [97], 22 out of 26 studies reported a statistically significant greater cIMT in obese and overweight children compared with the healthy control groups. As fat tissue is an active endocrine and immune organ whose dysfunction contribute to several pathologies [98], the obese and/or overweight patients also had higher values of serum lipids, inflammatory markers, blood pressure, or insulin resistance [97]. Though it is recognized that obesity, and in particular, abdominal obesity during childhood and adolescence is an important predictor for several CVD risk factors [99, 100], it is still unclear which measure of body composition or body size best predicts vascular wall changes in children. A couple of studies have addressed this issue using dual-energy radiographic absorptiometry (DXA) [101, 102] or even non-invasive ultrasound scanning [103] to measure body composition. However, the increasing sense of urgency for prevention of obesity in childhood prevention [104] demands simple, inexpensive measures of total and abdominal visceral fat tissue accumulation such as body mass index (BMI) and waist circumference (WC), respectively, as that may underscore a window of opportunity for interventions early in life.

Therefore, this study was designed to assess the differences in body composition measures among cIMT tertiles and to determine if general body mass, and body fat distribution in particular affects cIMT in 11-13 years-old children.

METHODS

The study population was sequentially studied between February and June 2012 without specific exclusion criteria other than been apparently healthy. Hence the investigation did not specifically target children who were overweight/obese, or of any particular fitness level.

Participants were 385 children (196 girls) aged 11 to 13 years-old from 6 schools of the Lisbon urban district. The study was approved by the Research Ethics Committees of the Faculty of Human Kinetics - University of Lisbon, Portugal. Informed consent was obtained from all subjects and their parents.

ANTHROPOMETRICS

Anthropometric measures were performed by a certified anthropometrist. Height and sitting height were measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg on a scale with an attached stadiometer (model 770, Seca; Hamburg, Deutschland) without shoes on. Leg length was calculated by subtracting sitting height from height. BMI was calculated as body mass divided by height squared ($kg.m^{-2}$). WC, an estimate of the subcutaneous and intra-abdominal fat tissue in the abdominal region [105] was measured to the nearest millimeter with an inelastic flexible metallic tape (Lufkin - W606PM, Vancouver, Canada) midway between the lower rib margin and the iliac crest, at the end of a gentle expiration. The mean value of the two closest measurements was used for analysis.

DUAL-ENERGY X-RAY ABSORPTIOMETRY

Total-body scans were performed by DXA and analyzed using an extended analysis program for body composition (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, Waltham, Massachusetts, USA) to determine total body fat (TBF) and trunk fat (TF). TBF was used as an estimate of total body fatness and TF was used as an estimate of a central pattern of fat (visceral + subcutaneous) distribution. The same technician positioned the subjects, performed the scans and completed the scan analysis according to the operator's manual using the standard analysis protocol. All scans were made in the morning after an overnight 12-hour fast. Quality control with spine

phantom was made every morning, and with step phantom every week. Repeated measurements with DXA in 18 young adults showed a coefficient of variation of 1.7% for TBF.

MATURITY

Maturity offset, that is, time before or after peak height velocity, was predicted with the equation of Mirwald et al. [106]:

*Maturity Offset in boys = -9.236 + 0.0002708*Leg Length and Sitting Height interaction - 0.001663*age and leg Length interaction + 0.007216*age and sitting height interaction + 0.02292*weight by height ratio*

*Maturity offset in girls = -9.376 + 0.0001882*leg length and sitting height interaction + 0.0022*age and leg length interaction + 0.005841*age and sitting height interaction -0.002658*age and weight interaction + 0.07693*weight by height ratio.*

Length measurements were made in centimeters and weight measurements are in kilograms. The weight by height ratio was multiplied by 100.

INTIMA-MEDIA THICKNESS

The cIMT was defined as the distance between the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall of the carotid artery.

Carotid ultrasound was performed on the right carotid artery using an ultrasound scanner equipped with a 25 mm linear 13 MHz probe (MyLab One, Esaote, Genova, Italy) and implemented with a previously validated radiofrequency-based tracking of arterial wall that allows a real-time determination of common carotid far-wall thickness (QIMT®) with high spatial and temporal resolution [88]. At present, automated edge-detection on the basis of RF signal processing of B + M mode US imaging is probably the most accurate method to detect cIMT [107].

Far-wall cIMT was automatically measured, and distension curves were acquired within a 1.59 cm region of interest in the common carotid artery, approximately 1 cm before the flow divider, where the operator places the region of interest. From the distension curves, maximum and minimum carotid diameters are obtained.

The coefficient of variation was 4.05% for cIMT and 2.71% for carotid diameter.

HEMODYNAMICS

The brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with the participants in the supine position using an automated oscillometric cuff (HEM-907-E, Omron, Tokyo, Japan). Two measurements were taken and if these values deviated by >5 mmHg, a third measurement was performed. The average of the two closest values was used.

STATISTICAL ANALYSES

All values were expressed as means and standard deviation. After categorizing subjects according to tertiles of cIMT [Low cIMT (LIMT): ≤0.46 mm; Middle cIMT (MIMT): 0.46-0.53 mm; High cIMT (HIMT): ≥0.53 mm), differences in body composition measures were assessed with ANOVA. Bonferroni test was used for post hoc comparison of means between each pair of groups. Furthermore, adjustment for potential confounding factors as BMI was performed using ANCOVA. ANOVA two-way was performed to test the influence of the interaction between sex and maturity on cIMT. Correlation analysis of the body composition data obtained from the entire sample with cIMT was calculated using Pearson correlation test. Using the r-to-z transformation, we calculated a value of z that was applied to assess the significance of the difference between the correlation coefficients. cIMT as dependent variable, and BMI, TBF, TF and WC as independent variables were analyzed in multiple linear regression analysis. The statistical significance level was $p < 0.05$.

RESULTS

Prevalence of overweight and obesity in this study was 28.31% according to the cut-offs for normal weight, overweight and obesity proposed by Cole & Lobstein [108]. When the subjects were divided in cIMT tertiles the highest relative prevalence of overweight and

obese children (in relation to the total number of participants in each tertile) was found in the HIMT group (39.23%) and the lowest in the MIMT group (22.04%) (FIGURE 2).

Taking into consideration the smoothed sex- and age-specific 85th percentile curve of WC estimated in the study of Sardinha et al. [109], 11.42% of the children in this study had high WC. The highest relative prevalence of high WC was found in the HIMT group (20%) and the lowest in the LIMT group (6.25%) (FIGURE 3).

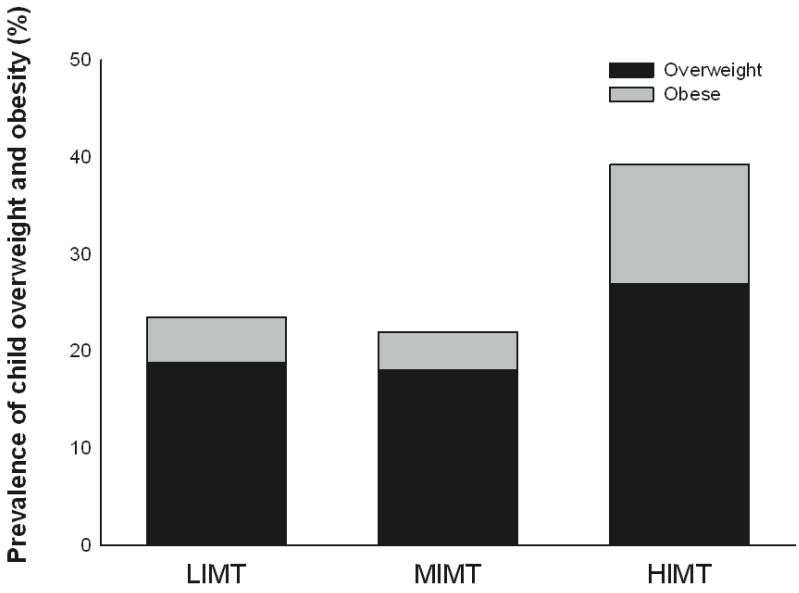

FIGURE 2: PREVALENCE OF CHILD OVERWEIGHT AND OBESITY BY TERTILES IN THIS STUDY ACCORDING TO THE CUT-OFFS FOR NORMAL WEIGHT, OVERWEIGHT AND OBESITY OF COLE ET AL. [108].

The three cIMT groups were similar for age, sex, maturity offset and DBP ($p>0.05$) (TABLE 1). Increasing cIMT was significantly associated with increasing degree of morphological characteristics. As compared with LIMT group, subjects in the HIMT group had higher mean values of weight, height, BMI, WC, TBF, TF, SBP and carotid diameter ($p<0.05$). When comparing LIMT to MIMT, the first had lower carotid diameter ($p<0.05$). The subjects in the HIMT group had also higher weight, height, BMI, TBF, TF, SBP and carotid

diameter in comparison with the MIMT group ($p<0.05$). Significant differences were found for WC even when controlling for BMI ($p<0.05$).

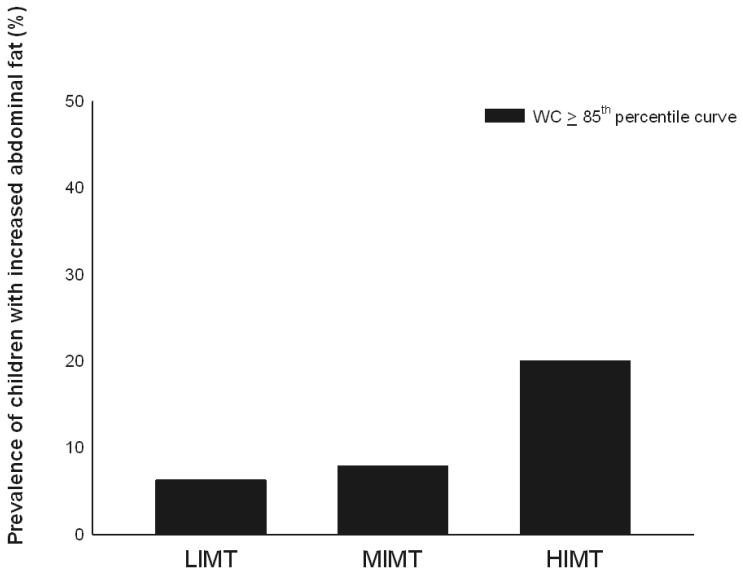

FIGURE 3: PREVALENCE OF CHILDREN WITH INCREASED ABDOMINAL OBESITY BY TERTILES IN THIS STUDY.

For the subsequent analysis, we first tested the influence of the interaction between sex and maturity on cIMT merging all subjects together. A non-significant contribution for sex and maturity was observed for cIMT ($p=0.08$; $p=0.06$, respectively).

cIMT was similarly correlated to BMI, TBF, TF and WC ($p<0.05$) with a trend for WC even if without significant difference between the correlation coefficients ($p>0.05$) (TABLE 2). The association between cIMT and TBF and TF was nearly identical (0.15).

Using multiple regression modeling with BMI, TBF, TF and WC, WC was the only independent predictor of cIMT level ($\beta=0.22$, $p<0.001$, $r^2=0.05$) (TABLE 3). TBF was the first ($p=0.81$) and TF was the last variable to be excluded from the model ($p=0.11$).

TABLE 1: MEANS (± STANDARD DEVIATION) OF THE CHARACTERISTICS OF THE STUDY GROUP AND DIFFERENCES BETWEEN IMT TERTILES.

		LIMT	MIMT	HIMT	All
n		128	127	130	385
Boys/Girls		62/66	63/64	64/66	189/196
Age	(years)	11.35 ± 0.55	11.50 ± 0.60	11.43 ± 0.58	11.43 ± 0.7
Weight	(Kg)	42.63 ± 8.37	43.93 ± 9.40	48.70 ± 12.20*†	45.11 ± 10.44
Height	(cm)	149.92 ± 6.56	151.06 ± 7.46	153.64 ± 8.21*†	151.55 ± 7.59
BMI	(kg.m-2)	18.87 ± 2.95	19.11 ± 3.04	20.45 ± 4.02*†	19.48 ± 3.44
WC	(cm)	65.15 ± 5.97	66.01 ± 6.76	69.23 ± 8.63*†	66.81 ± 7.41
TBF	(Kg)	11.93 ± 4.86	11.79 ± 5.67	14.38 ± 7.42*†	12.71 ± 6.19
TF	(Kg)	4.39 ± 2.21	4.38 ± 2.74	5.55 ± 3.57*†	4.78 ± 2.94
Maturity	(years)	-0.98 ± 1.06	-0.82 ± 1.04	-0.77 ± 1.14	-0.86 ± 1.08
SBP	(mmHg)	109.90 ± 10.02	110.87 ± 11.04	113.39 ± 11.09*	111.40 ± 10.80
DBP	(mmHg)	61.56 ± 8.08	61.34 ± 8.61	62.42 ± 7.76	61.78 ± 8.15
cIMT	(mm)	0.41 ± 0.03	0.49 ± 0.01	0.58 ± 0.05*#†	0.49 ± 0.08
Diameter	(mm)	6.13 ± 0.51	6.25 ± 0.42	6.39 ± 0.49*	6.26 ± 0.49

* Significant differences between LIMT and HIMT ($p<0.05$); # Significant differences between LIMT and MIMT ($p<0.05$); † Significant differences between MIMT and HIMT ($p<0.05$)

DISCUSSION

We assessed the differences in body composition measures among cIMT tertiles and determined which measure of body composition exhibited the greatest association with cIMT in 11-13 years-old children. The main findings are that 1) differences exist in body composition variables among cIMT tertiles and 2) significant correlations were found between cIMT and BMI, TBF, TF and WC, yet in the final model 3) only WC was a significant predictor of increased cIMT in 11-13 years-old children. This result was further strengthened after adjustment for BMI.

The prevalence of overweight and obesity in this study was 28.31% according to the cut-offs for normal weight, overweight and obesity of Cole & Lobstein [108]. This was similar to the 26.7% assembled for this age range from the work of Sardinha et al. [110] in a representative sample of Portuguese youth aged 10-18 years-old. Nearly 50% of the overweight and obese children in this study were in the HIMT group. As in several previous studies [34, 93, 94] these results showed that obese and overweight children exhibit

significantly increased cIMT when compared with the non-obese children of similar age, supporting the understanding that the onset of the vascular subclinical changes occur at an early age in obese children.

TABLE 2: PEARSON CORRELATION BETWEEN cIMT AND BMI, TBF, TF AND WC AND VALUE OF Z BETWEEN WC AND BMI, TBF AND TF.

cIMT	r	p	Z	p
BMI	0.188	<0.001	-0.47	0.63
TBF	0.153	0.003	-0.97	0.32
TF	0.155	0.002	-0.94	0.34
WC	0.221	<0.001	---	---

Overweight and obese children and adolescents are usually identified using BMI as an index of general fatness. However, it has been suggested that BMI may be a less sensitive indicator of fatness amongst children and adolescents than WC or waist-to-height ratio [111]. Hence, we further calculated the prevalence of large WC among the cIMT tertiles. Mean WC in this sample was 66.81 cm, consistent with the 68.85 cm previously shown for this age range in a school-based study performed in Portugal with a total of 22003 children and adolescents aged 10-18 years-old [109]. Among the 385 children enrolled in the present study, high WC was present in 11.42% according to the estimated smoothed sex- and age-specific 85th percentile curve of WC. More than 50% of the children with high WC were in the HIMT group. These results are consistent with the evidence that abdominal obesity during childhood and adolescence is an important predictor for several CVD risk factors [99, 100]. Additionally, abdominal obesity is a critical component of the pediatric metabolic syndrome definition proposed by the International Diabetes Federation [112]. WC is a good correlate of subcutaneous and total trunk fat across a large spectrum of BMI and may also serve as a surrogate for markedly enlarged visceral fat depots in obese children [109]. Post-hoc analysis in the present study showed that TF and WC were highly correlated ($r=0.92$; $p<0.001$).

TABLE 3: MULTIPLE STEPWISE LINEAR REGRESSION ANALYSIS, WITH MEAN cIMT AS THE DEPENDENT VARIABLE.

	β	95% Confidence Interval		*t*	*p*	r^2
WC	0.31	0.00	0.01	2.2	0.03	
BMI	0.60	-0.01	0.01	0.39	0.70	
TBF	-0.74	0.00	0.00	-0.24	0.81	0.58
TF	-0.09	0.00	0.00	-0.29	0.77	
SEX	0.16	-0.02	0.02	0.27	0.78	
AGE	0.49	-0.01	0.02	0.96	0.34	
WC	0.31	0.00	0.01	2.25	0.03	
BMI	0.46	-0.01	0.01	0.32	0.75	
TF	-0.15	0.00	0.00	-1.11	0.26	0.58
SEX	0.17	-0.02	0.02	0.29	0.77	
AGE	0.05	-0.01	0.02	0.96	0.34	
WC	0.33	0.00	0.01	2.54	0.01	
BMI	0.05	-0.01	0.01	0.33	0.74	0.58
TF	-0.17	0.00	0.00	-1.40	0.17	
AGE	0.05	-0.01	0.02	0.97	0.33	
WC	0.35	0.00	0.01	3.37	0.00	
TF	-0.15	0.00	0.00	-1.43	0.15	0.58
AGE	0.05	-0.01	0.02	0.94	0.35	
WC	0.37	0.00	0.01	3.57	0.00	
TF	-0.17	0.00	0.00	-1.62	0.11	0.55
WC	0.22	0.00	0.00	4.44	0.00	0.49

Previously reported values for mean cIMT in pediatric subjects show a wide variation from 0.32 mm [113] to 0.64 mm [43], while other studies report values in-between [36, 85]. Values measured in obese Chinese children [114] and in hypertensive and obese North American children [43] ranged above 0.60 mm, the latter with an upper quartile > 0.80 mm. In our study the mean cIMT was 0.49 mm though the mean cIMT of the HIMT group, that had the largest prevalence of overweight and obese children, was 0.58 mm (range for HIMT group: 0.52 - 0.74 mm), which is within the values reported in the literature for these particular phenotypes. To become aware that the highest cIMT in our study was 0.74 mm, 0.45 mm higher than the lowest cIMT (range for LIMT group: 0.30 – 0.46 mm) and similar to what has been reported as the cIMT of 67.4 ± 7.6 years-old healthy seniors (0.77 mm) [12] is in fact disturbing. These numbers should be taken into account when putting together public health policies aiming at reducing the severity and extent of coronary artery disease and strokes in adults as arterial wall changes are a continuous in vivo process [12] with increased cIMT already been demonstrated in obese and overweight children.

A 0.17 mm difference in cIMT between LIMT and HIMT groups suggest differences of 4.08 cm in WC, 1.58 $Kg.m^{-2}$ in BMI, 2.45 and 1.16 Kg in TBF and TF respectively and 3.49 mmHg in SBP ($p<0.05$). This is disconcerting considering that it is very difficult to reduce cIMT through interventions. Woo et al. [63] showed that dietary and/or exercise intervention programs in 82 overweight children (BMI, 25±3), 9-12 years-old, who were randomly assigned to dietary modification only or diet plus a supervised structured exercise program for 6 weeks and subsequently for 1 year, produced only small changes in cIMT. It took 6 weeks to the exercise plus diet group to have a 0.01 mm reduction (0.47 to 0.46 mm) in cIMT and after 1 year the group that continued training and the group that dieted only had a 0.02 mm difference (0.48 to 0.46 mm; 0.47 to 0.45 mm, respectively) to the cIMT baseline values, the first with a 4.9% change in %TBF although with no significant waist-to-hip ratio difference. Thus, even with a large reduction in body fat, only minimal changes in cIMT were observed.

Accumulation of fat mass in the abdominal area increases the risk of metabolic complications such as diabetes, dyslipidemia, hypertension and atherosclerosis, which are associated with a high morbidity and mortality [115]. A relation between central fat accumulation and the development and progression of carotid atherosclerosis has been established [116, 117] and concluded that atherosclerotic events could be avoided by preventing abdominal obesity. Our univariate analysis showed a significant correlation between cIMT and WC. Although this correlation was not significantly different from the correlation obtained between cIMT and BMI, TBF, and TF, our multiple linear regression analysis showed that WC was the only independent determinant of cIMT. Some risk factors have an increased impact on cIMT with aging, and these appear to be more associated with the progression of atherosclerosis [118], while others including BMI and presumably WC have a relatively stable association with cIMT across age, supporting the use of WC measurement since early childhood.

Elkiran et al. [94] also found a statistically significant correlation between cIMT and WC measurement in both univariate and multivariate analysis in a study of 67 obese and 24 overweight children (13.3±0.86 years-old; 13.2±0.69 years-old, respectively). In the study of Fang et al. [96] including 46 boys and 17 girls with a mean age of 10.5 ± 1.6 years-old (BMI Obese group: 28.04 $Kg.m^{-2}$), cIMT was correlated to weight, BMI, WC, waist-to-hip ratio, SBP, DBP and other biochemical variables. However, from the morphological

variables assessed, only WC and waist-to-hip ratio were independent determinants of mean cIMT level. Morrison et al. [85] examined the influence of demographic, family history, anthropometric characteristics and traditional cardiovascular risk factors on cIMT, assessing cIMT in 148 children 5-16 years-old (mean age: 11 years-old). Of the risk factors where sufficient data was collected (dysglycemia, hypertension, low fruit and vegetable intake, abdominal obesity and low physical activity), cIMT was related to WC, BP and insulin resistance in univariate regression. Alpsoy et al. [119] measured cIMT in overweight (n = 67) and normal weight children (n = 115, controls) 6-15 years-old and concluded that age, WC, and serum triglycerides were independent predictive risk factors for increased vascular structure. Just recently, Geerts et al. [103] with a sample of 306 5-year-old children from an on-going birth cohort reported that increased intra-abdominal fat measured by ultrasound was related to thicker cIMT, and increased WC with thicker cIMT, lower distensibility, and higher elastic modulus, independent of BMI. However, our study is the first to show the effect of increasing BMI, TBF and TF but especially of increasing WC on cIMT in a large sample of 11-13 years-old children and that maturity, sex and age did not influence this relationship. Our findings indicate that fat localization in children may be a better risk marker than overall measures of body fat such as BMI. Despite increasing interest in the measurement of regional fat distribution, one difficulty with this area of research is the inexistence of commonly accepted cut-offs for classifying subjects with high central pattern of fat distribution regardless of assessment method. However, age- and sex-specific WC reference data for Portuguese children and adolescents aged 10-18 years have been estimated [109], providing useful information for abdominal risk assessment and clinical and lifestyle intervention.

Much variation in body size as well as in vasculature may be a function of varying growth rates of children. Accordingly, the results of the present study have to be interpreted recognizing this possibility. In post hoc analysis we found height to be associated with cIMT ($r=0.20$, $p<0.001$) in conformity with other studies [52, 103, 120], which may have influenced the multivariate regression analysis in favor of WC, which is assumingly less dependent on height than TF. Actually, TF was no longer associated with cIMT after controlling for height ($p=0.06$). Nevertheless, WC was found to correlate similarly with fatness-related biologic factors compared with TBF and TF [121]. For geometrical reasons it is conceivable that both the arterial diameter and wall thickness need to adapt in order to

maintain, or even increase, blood flow through the lengthening arterial tree [52]. cIMT was also correlated with SBP ($r=0.14$, $p<0.05$) but not when adjusted for height ($r=0.06$; $p=0.21$). Because mean levels of WC and TF are highly correlated (0.88, $p<0.001$), it could be argued that the cIMT-WC association simply may be due to chance (colinearity with TF). However, at least three observations support the view that cIMT and WC are related independent of TF. First, if we divide the cohort in two groups according to their TF level, the significant associations between WC and cIMT were still observed within the highest TF group. Second, when the influence of TF was partialled out, the relationship between cIMT and WC in the pooled population remained significant ($r=0.18$; $p<0.001$). Third, when subjects with higher TF were divided into lower and higher WC groups, the higher WC group had greater cIMT than the TF-matched lower WC group ($p<0.05$).

This study has several strengths and limitations. Automated edge-detection on the basis of RF signal processing of B + M mode US imaging is probably the most accurate method to detect cIMT [107]. The large number of participants in this study consent increased representativeness. This was a cross-sectional study, thus no cause-effect can be inferred nor confirm the speculations made. The tertiles set by the authors may not predict risk of atherosclerosis at all, but as far as we know there is no considerable normative data in this age group. The multiple regression analysis explained only a small portion of the variance in cIMT, suggesting that in addition to WC, other variables, conceivably physical activity objectively measured and cardiorespiratory fitness among others, are involved in determining cIMT, but this was not tested in our study. Abdominal fat mass derived from DXA was not used in the statistical analysis. It is our opinion that observational, cross-sectional studies comparing correlations between MRI-measured total visceral adipose tissue and surrogate measures including DXA ROIs are needed in children before we started using abdominal ROI from DXA as estimates of visceral fat as with WC.

CONCLUSION

Differences exist in body composition variables among cIMT tertiles. Waist circumference was the only significant predictor in the multivariate model in these 11-13 years-old children. Accordingly, even moderate degrees of regional fatness influenced arterial structure and are related to cardiovascular risk. Taking into account the increases in overweight and obesity among children internationally over the past three decades [122],

this study supports public health policies aiming at the prevention of obesity and central fat patterning early in life in order to improve vascular health.

INSIGHTFUL DISCUSSIONS WITH PEERS

Typically cIMT is measured in end-diastole or the corresponding R-wave. Can the authors provide a reason as to why this wasn't done? Please refer to the paper: Changes in cIMT during the cardiac cycle: The multi-ethnic study of atherosclerosis by Polak et al. [123].

According to Hoeks et al. [124], in wall track systems, the received radiofrequency ultrasound signals over a large number of observations are analyzed. To allow for proper identification of the vessel walls the wall track system (MyLab One, Esaote, Genova, Italy) stores the radiofrequency signals over 6 heart beats, consequently much more accurate than one single measurement in a certain phase of the heart cyclus (e.g. end-diastole). Each mean value is the mean of up to 45 measurements of cIMT per frame calculated on the far wall in the 15 mm length of the common carotid. Furthermore, the system indicates the standard deviation of these 6 mean values. Only the data with a standard deviation <21 μm are accepted. Without averaging the measurements, the intra-subject error would be much higher, also for trained sonographists. The user has to identify the position of the vessel walls as part of the procedure to detect the displacement of vessel walls and, hence, the change in diameter as a function of time. Therefore, it requires only a minor modification to store the data within a window of 3 mm around the blood wall interface on the posterior side of the artery. Because pressure changes are most pronounced in the early phase of the cardiac cycle, processing is restricted to 500 ms from the R-peak. Although, for radiofrequency line alignment, each radiofrequency line acquired at a sample rate exceeding 500 Hz, has to be processed, amplitude averaging is indicated at a much lower time spacing because of the low speed of tissue displacement. To reduce computational complexity, averaging is only executed at intervals of 50 ms, whereas displacement detection is performed for each line.

The tertiles set/divided by the authors may not be at risk at all. I can understand that there may not be considerable normative data in this age group. Hence, have the authors tried to divide the cIMT measurements based on the ARIC study. Based on that study, only the 75th percentile and above (for age and sex matched) are considered at risk. It would be better if the authors can provide a reason not diving the participants based on that scale.

The early work of Howard et al. [125] provided a description of the distribution of carotid atherosclerosis in the general 45-64 years-old population by race-sex strata. We believe that if we had adopted the 75% percentile we would be equally questioned of its adequacy in children. Yet, we clearly understand the need for standardizing cut-offs when predicting risk of atherosclerosis. Most cross-sectional and interventional studies on cIMT in children have outlined this issue by comparing obese or overweight children with lean controls using known cut-offs for overweight and obesity. Jourdan et al. [52], although with a limited sample size, provided age- and height-related reference tables, which permit appropriate normalization of values measured in adolescents. We intentionally choose a methodological design where graded relations between cIMT and body compositions phenotypes could be shown making this study distinct from any other published so far.

Is it possible that the reason WC was the only significant predictor in the regression model is due to the fact that many of the body composition variables (including TF and WC) were highly correlated?

Because mean levels of WC and TF are highly correlated (0.88, $p<0.001$), it could be argued that the cIMT-WC association simply may be due to chance (colinearity with TF). However, at least three observations support the view that cIMT and WC are related independent of TF. First, if we divide the cohort in two groups according to their TF level, the significant associations between WC and cIMT were still observed within the highest TF group. Second, when the influence of TF was partialled out, the relationship between cIMT and WC in the pooled population remained significant ($r=0.18$; $p<0.001$). Third, when subjects with higher TF were divided into lower and higher WC groups, the higher WC group had greater cIMT than the TF-matched lower WC group ($p<0.05$). Besides, collinearity is a statistical phenomenon in which two or more predictor variables in a multiple regression model are highly correlated. We only got one predicted variable, so in

this situation the coefficient estimates are not changing erratically in response to small changes in the model.

It seems to me inappropriate to combine girls and boys into one group.

We understand the point of the reviewer and it has in fact been addressed in the manuscript by testing the influence of the interaction between sex and maturity on cIMT. A non-significant contribution for sex and maturity was observed for cIMT ($p=0.08$; $p=0.06$, respectively) and cIMT was not significant different between sexes ($p>0.05$). However, we found significant differences between sexes for maturity, TBF and TF ($p<0.001$). Yet, BMI and WC were not significant different between boys and girls ($p=0.08$, $p=0.71$, respectively). As the latter was the only independent predictor of cIMT in multivariate regression analysis we consider appropriate to generalize the results for both sexes. This option is well supported in literature with a wide range of publications opting for the same statistical procedure [64, 85, 93, 94, 96, 103, 113, 126, 127].

5. SINGLE AND COMBINED INFLUENCES OF BODY COMPOSITION PHENOTYPES ON CAROTID INTIMA-MEDIA THICKNESS IN CHILDREN

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ABSTRACT

BACKGROUND: Central fatness has been shown to be a more sensitive predictor of atherosclerotic changes in the carotid artery in children than are total body fat measures. However, it is unclear whether a total body fat measure coupled with an estimate of a more central pattern of fat accumulation predicts increased carotid intima-media-thickness (cIMT) better than either measure alone.

OBJECTIVE: To identify the ability of a combination of simple anthropometric screening tools or a combination of objective measures of body composition to predict cIMT.

METHODS: cIMT was assessed in the common carotid artery in 349 children aged 11-12 years-old (183 girls). Body mass index (BMI), waist circumference (WC) and waist-to-height ratio (WHtR) were dichotomized according to established criteria and indices of total body (TBFI) and abdominal (ABFI) fat were assessed by DXA and categorized into normal and increased risk (>85%). Single and combined associations among anthropometric and laboratorial body composition measures with the risk of having increased cIMT (>85%) and discriminatory performance were tested with logistic regression analysis and ROC analysis.

RESULTS: Children with higher total fatness (BMI and TBFI) or higher central pattern of fat accumulation (WC, WHtR and ABFI) were in higher risk for increased cIMT (OR: 2.08-3.24). The risk for increased cIMT was not higher among children who coupled high total and high central fatness (OR: 2.27-3.10).

CONCLUSIONS: Combination of total body fat assessment and central pattern of fat accumulation measures does not improve the prediction of increased cIMT in children.

Simple surrogate measures of fatness can be used to predict increased cIMT urging for special attention to those children who exhibit an increased central body fat phenotype.

KEYWORDS:

Children; Intima-Media Thickness; Body Mass Index; Waist Circumference; Ratio; DXA; Coupled

INTRODUCTION

Childhood growth is associated with an expansion of the intra-abdominal adipose tissue depot [128] and several indices of abdominal obesity are associated with common cardiovascular disease risk factors among children and adolescents [129-132]. Waist circumference (WC) or waist-to-height ratio (WHtR) have been suggested as the best anthropometric variables when predicting cardiovascular disease risk assessed by traditional risk factors such as total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and systolic and diastolic blood pressure [130, 131]. Ultrasound imaging of carotid intima-media thickness (cIMT), a recognized intermediate phenotype for early atherosclerosis and an important predictor of future vascular events in adults [133] is also associated with WC and WHtR [94, 103, 134]. It has been suggested as well that the development and progression of carotid atherosclerosis into adulthood could be avoided by preventing abdominal obesity in youth [117].

There seems to be no consensus on whether total or abdominal indices of fatness should be used preferably as non-invasive markers of obesity-related cardiovascular disease risk. Recently it has been suggested that both BMI and WC should be measured in clinical practice considering the latter as a vital sign in cardiology [135]. However, BMI and WC or WHtR may not differ in their ability to identify children with adverse risk factors [136]. We have previously shown that WC was the only independent determinant of cIMT [134] but the correlation coefficients between cIMT and WC were not significantly different from those obtained between cIMT and BMI and other laboratorial measures such as total body fat (TBF) and trunk fat assessed by dual-energy X-ray absorptiometry (DXA).

However, some studies bring into question the potential utility of a combination measure between WC and BMI in the prediction of traditional cardiovascular disease risk factors [137, 138]. Children with high abdominal fatness within the overweight BMI category were ~2 times more likely to have high triglyceride levels, high insulin levels, and the metabolic syndrome, compared with the low WC group [137]. Less is known about the combined influence of BMI and WC among children and adolescents on more specific arterial risk factors such as cIMT. Although the above-mentioned studies indicate that combined measures of total and central fatness may provide a better overall prediction of cardiovascular disease risk factors it is unclear whether this combination predicts cIMT, better than either measure alone. Therefore, this study sought to identify the ability of a combination of simple anthropometric screening tools or a combination of objective measures of body composition to predict increased cIMT in children.

METHODS

Participants were 349 children (183 girls) between 11 to 12 years of age, enrolled in 2012 from 6 schools of Portugal. The ethics committee of the Faculty of Human Kinetics - University of Lisbon approved this study. Children provided assent for their participation and informed consent was obtained from their legal guardians.

The study population was sequentially studied between February and June 2012 without specific exclusion criteria other than being apparently healthy. Hence the investigation did not specifically target children who were overweight/obese, or of any particular fitness level.

ANTHROPOMETRY

Anthropometric measurements were performed by a certified anthropometrist. Height was measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg on a scale with an attached stadiometer (model 770, Seca; Hamburg, Deutschland) wearing minimal clothing and no shoes. BMI was calculated as body mass divided by height squared ($kg.m^{-2}$) and categorized into normal weight, overweight, or obese according to the WHO standards [139]. WC was measured to the nearest millimeter with an inelastic flexible metallic tape (Lufkin - W606PM, Vancouver, Canada) at the iliac crest level. The

mean value of the two closest measurements was used for analysis. Age- and gender-specific WC percentiles for youths developed in a Portuguese nationally representative sample [109] were used to dichotomize WC into normal (<P90) or increased risk (≥P90).

Because WC and BMI depend on the use of sex- and age-specific percentile tables in children, waist-to-height ratio (WHtR) has emerged as more attractive option [140], and several studies of children have concluded that this ratio is more strongly associated with cardiovascular disease risk factors than is BMI [131]. WHtR was calculated [WC (cm) / height (cm)] and dichotomized into normal (≤0.5) and increased risk (>0.5).

DUAL-ENERGY X-RAY ABSORPTIOMETRY

Total-body scans were performed by DXA (Explorer W, Hologic; Waltham, MA, USA) and analyzed using the equipment's software (QDR 12.4, Waltham, MA, USA) to determine TBF and lean soft tissue (TBLST=TB lean mass–TB bone mineral content). Repeated measurements with DXA in 10 young adults showed a coefficient of variation (CV) of 1.7% for TBF and 0.8% for TBLST. All scans were submitted to additional analysis by region of interest to assess fat content on the abdominal region (ABF; CV=0.01%). The upper and lower limits of the abdominal region of interest were determined as the upper edge of the second lumbar vertebra and the lower edge of the fourth lumbar vertebra [141] at the top level of the iliac crests, respectively. The side limits were determined as to include all trunk length, but exclude any upper limb scan area. The same technician positioned the subjects, performed the scans and completed the scan analysis according to the operator's manual using the standard analysis protocol. All scans were made in the morning after an overnight 12-hour fast. Quality control with spine phantom was made every morning, and with step phantom every week.

The TBF index (TBFI), ABF index (ABFI) and TBLST index (TBLSTI) were calculated through the normalization of TBF, ABF and TBLST for height. Increased risk was defined as ≥85th percentile (P85) for age and sex.

INTIMA-MEDIA THICKNESS

cIMT was defined as the distance between the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall of the right carotid artery. The artery was imaged with an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy) using previously validated radiofrequency-based tracking of the arterial wall that allows a real-time determination of common carotid far-wall thickness (QIMT®) with high spatial and temporal resolution. cIMT was automatically measured, and distension curves were acquired within a segment of the carotid artery about 1 cm before the flow divider, where the operator places the region of interest. The coefficients of variation for repeated measurements in our laboratory was 4.05% for cIMT and 2.71% for carotid diameter [134]. Increased cIMT was defined as ≥ P85 for age and sex.

HEMODYNAMICS

The brachial systolic (SBP) and diastolic (DBP) blood pressure were measured with the participants in the supine position using an automated oscillometric cuff (HEM-907-E, Omron, Tokyo, Japan). Two measurements were taken and if these values deviated by >5 mmHg, a third measurement was performed. The mean value of the two closest measurements was used. Pulse pressure (PP) was calculated for adjustment purposes as follows: PP=SBP-DBP.

STATISTICAL ANALYSES

All values were expressed as means and standard deviation unless otherwise noticed. Subjects were placed into categories of total (BMI, TBFI and TBLSTI) and central (WC, WHrt and ABFI) body composition phenotypes as described. ANOVA and ANCOVA were used to compare the descriptive characteristics of the participants and cIMT within each single and combined category (BMI+WC, BMI+WHtR, TBFI+ABFI). Pulse pressure (PP) and the diameter of the carotid artery were used as covariates.

Sex- and PP-adjusted logistic regression analyses were used to examine the single and combined effects of categories of body composition phenotypes on increased cIMT. Additional adjustments to TBLSTI were performed in TBFI and ABFI analysis. The low body composition phenotypes were used as the reference groups (OR: 1). The

probabilities were saved for the single and combined effects of categories of body composition phenotypes on cIMT, and sex-adjusted ROC curve analysis were conducted next to determine the performance of single and combined body composition phenotypes in the discrimination of increased cIMT.

The statistical analyses were computed and analysed by a certified researcher using the SPSS Statistics 21 and MedCalc 11.1.1.0. Significance was set at $p<0.05$.

RESULTS

The descriptive characteristics of the participants are presented in TABLE 4. Thirty-four percent of the children in this study were overweight or obese according to de Onis et al. [139] and 28% according to Cole et al. [142]. Significant differences were found in all variables from TABLE 4 between BMI categories. The prevalence of children with high central pattern of fat accumulation ranged from 15% (WC) to 20% (WHtR).

TABLE 4: MEANS AND SD OF THE CHARACTERISTICS OF THE STUDY GROUP.

		Normal Weight	Overweight/Obese	All
n		231	118	349
Age	(years)	11.39 ± 0.49	11.25 ± 0.44*	11.34 ± 0.48
Girls/Boys	(n)	115/116	68/50	183/166
BMI	($kg.m^{-2}$)	17.53 ± 1.56	23.36 ± 2.95*	19.50 ± 3.49
WC	(cm)	64.82 ± 4.93	78.86 ± 8.83*	66.79 ± 7.39
WHtR		0.43 ± 0.28	0.51 ± 0.05*	0.46 ± 0.06
TBF	(%)	24.03 ± 5.35	34.62 ± 6.70*	27.61 ± 7.70
TBFI	($kg.m^{-2}$)	4.23 ± 1.18	8.13 ± 2.46*	12.81 ± 6.19
ABFI	($kg.m^{-2}$)	0.26 ± 0.14	0.65 ± 0.29*	0.91 ± 0.65
LSTI	($kg.m^{-2}$)	12.66 ± 1.21	14.35 ± 1.25*	13.23 ± 1.46
SBP	(mmHg)	108.57 ± 9.57	115.16 ± 10.38*	110.76 ± 10.37
DBP	(mmHg)	60.30 ± 7.62	63.72 ± 8.51*	61.47 ± 8.15
Diameter	(mm)	6.19 ± 0.48	6.39 ± 0.51*	6.26 ± 0.50

*Significant difference from normal weight group ($p<0.05$).

When the remaining body composition phenotypes (TBFMI, ABFMI and TBLSTI) were dichotomized and examined individually, children from all high-risk categories had increased cIMT (+0.03 mm to +0.04 mm; p<0.05; FIGURE 4) except between TBLSTI categories. Adjustments for the diameter of the carotid artery and PP did not attenuate the differences, which remained significant. The sex-adjusted ORs were comparable in magnitude although the highest OR for increased cIMT was found in participants with increased WC compared to the normal WC group (FIGURE 6). Adjustment for PP increased the OR in all variables. TBLSTI had no predictive ability of increased cIMT (p=0.764) and did not affect significantly the OR in the TBFI analysis. The potential of the single effect of body composition phenotypes to discriminate increased cIMT was tested and found higher than would be expected by chance (TABLE 5). The highest area under the curve (AUC) was found for WC.

TABLE 5: PERFORMANCE OF SINGLE AND COMBINED BODY COMPOSITION PHENOTYPES IN THE DISCRIMINATION OF INCREASED cIMT THROUGH SEX-ADJUSTED RECEIVER OPERATING CHARACTERISTIC CURVES ANALYSIS

	BMI	WC	WHtR
BMI	0.636 (0.0449)* 72.0 / 51.5	0.674 (0.0442)* 56.0 / 77.3	0.637 (0.0448)* 70.0 / 53.5
WC		0.674 (0.0442)* 86.0 / 44.8	
WHtR			0.610 (0.0451)* 42.0 / 80.3

	TBFI	ABFI	TBLSTI
TBFI	0.608 (0.0451)* 38.0 / 84.6	0.606 (0.0451)* 38.0 / 84.3	0.617 (0.0450)* 40.0 / 80.6
ABFI		0.613 (0.0451)* 38.0 / 82.6	0.625 (0.0450)* 52.2 / 72.2
TBLSTI			0.591 (0.0451)* 54.0 / 67.2

Data within each cell are: area under the curve (standard deviation) plus Sensitivity/Specificity; * Significant at p<0.05

The prevalence of children combining increased total body and abdominal fatness ranged from 12.6% (TBF and ABF) to 16.6% (BMI and WHtR). Participants with both high total body fatness (BMI, TBFI) and high central fat accumulation (WC, WHtR, ABFI) or only one of these increased had cIMT>P85 (+0.03 mm to +0.04 mm; p<0.05). Differences remained significant after adjustments for the diameter of the artery and PP. Significant sex-adjusted

OR for increased cIMT were found when one or both combined variables were increased. Adjustment for PP increased the OR in all variables but additional adjustments for TBLSTI removed the significance of the combination TBFI+ABFI (p=0.143). The highest OR for increased cIMT was found among children who were overweight or obese and had high WC compared to those with normal body fat phenotype. The highest potential to discriminate increased cIMT came from combined effect of BMI+WC (TABLE 5).

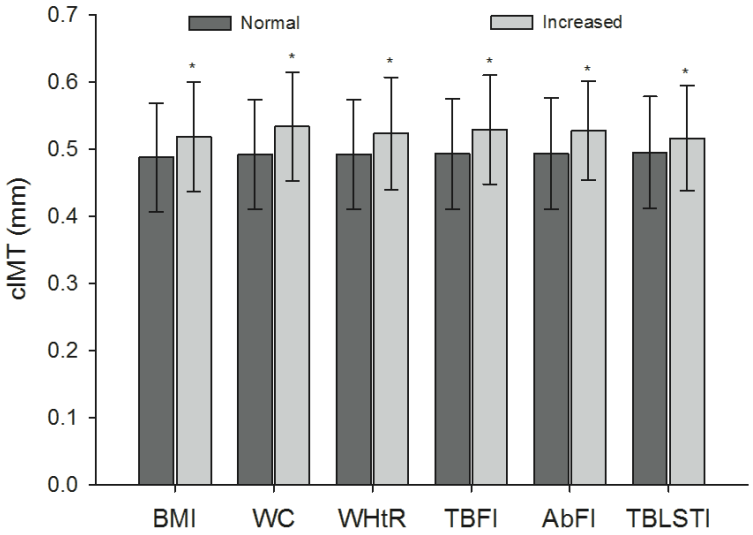

FIGURE 4: CIMT ACCORDING TO CENTRAL PATTERN OF FAT ACCUMULATION GROUPS AND TOTAL BODY FAT MASS CATEGORIES.

* Significant difference from normal category

DISCUSSION

We provide evidence that a combination of total body fat assessment coupled with central pattern of fat accumulation measures does not improve the prediction of increased cIMT in children. Single anthropometric and objective measures of body composition were useful in identifying children at risk, with emphasis on the predictor potential of WC for increased cIMT. As suggested for adults [135], the present results highlight the need to measure abdominal fatness in pediatric practice as a useful and vital sign that is associated with vascular health.

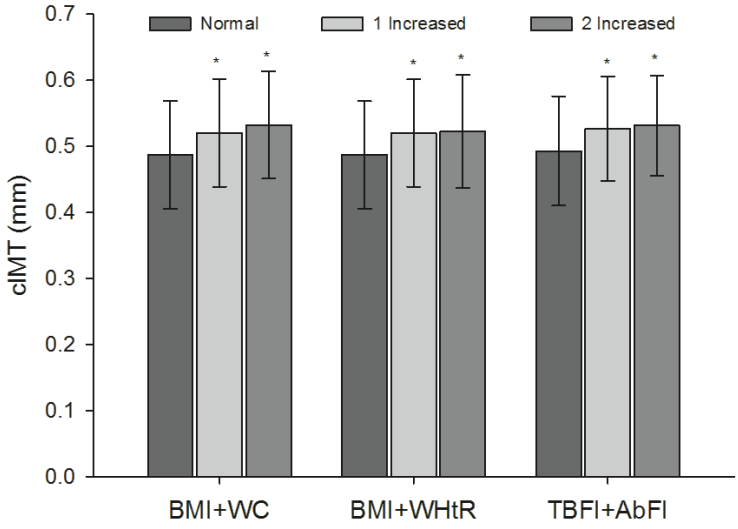

FIGURE 5: CIMT IN THE COMBINED CENTRAL PATTERN OF FAT ACCUMULATION GROUPS AND TOTAL BODY FAT MASS CATEGORIES.

* Significant difference from normal category

An increased BMI from 10 years of age is considered a predictor of premature death by acute myocardial infarction during adult life [143]. However, obesity-related comorbidities seem to be more closely associated with patterns of body fat accumulation than with the amount of total body fat [144]. WC related indices were the only independent obesity-related predictors of early subclinical manifestations of cardiovascular pathology [103, 134].

Our results show that there is little difference in the ability of surrogate measures of total and abdominal fatness to identify cardiovascular risk in children, in agreement with others [137, 145]. However, the highest OR for increased cIMT was found in children with high WC compared to the normal WC group, though all the OR for increased cIMT were comparable in magnitude, except for TBLSTI. While recognizing that cross-sectional associations do not amount to causality, it is conceivable that the differences in cIMT between body composition phenotypes were not due to differences in the metabolically active tissue between the groups as previously reported [146].

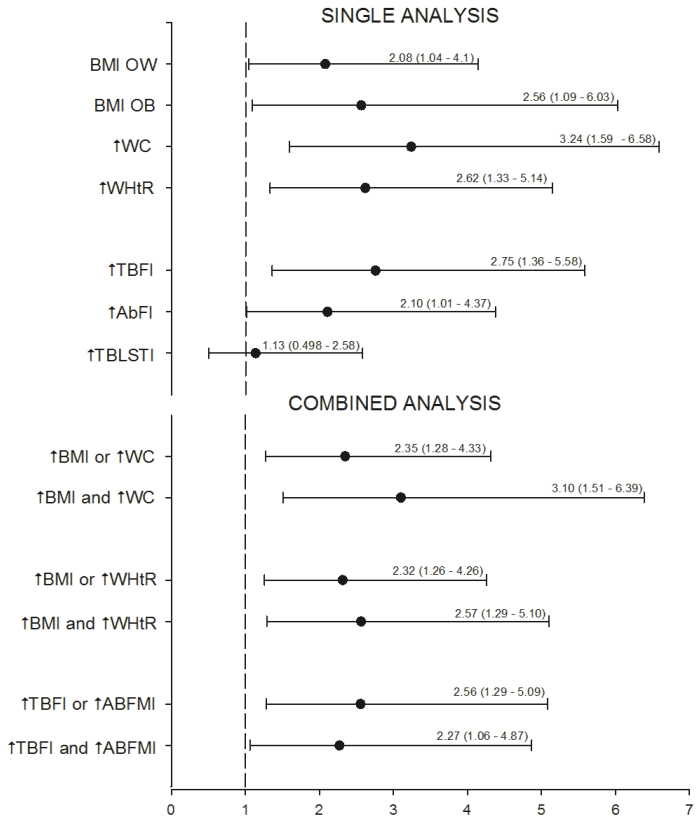

FIGURE 6: ORS FOR INCREASED CIMT WHEN BODY COMPOSITION VARIABLES WERE TESTED INDIVIDUALLY OR COMBINED.

Arrow in tick label denotes “increased” as described in the methods
Values are mean (95% CI)

The risk of increased cIMT was also evident in the high WHtR category. This is important as WHtR is an attractive and practical option because a single cutoff (0.5) can be used among both children and adults [140]. For this reason, some authors suggest that WHtR may be preferred as an indicator of obesity-related risk even if the predictive abilities of WHtR and BMI are similar [131, 140]. In addition, it is possible that WC related indices are more appropriate in children than BMI, given that these measures also encompass subcutaneous fat, which has been suggested to have a role in insulin resistance in children with small amounts of visceral fat [147]. Despite some disagreement [148], cardiovascular risk factors tend to group together more frequently above a WHtR of 0.5 and our results show that the OR for increased cIMT among children with WHtR above 0.5 were 2.6 times larger than the odds for children with low WHtR.

Some studies have tried to determine whether using a combination of BMI and WC related indices is clinically helpful in identifying children with high metabolic and cardiovascular risks. In a cohort of 1479 children aged 5 to 15 years, overweight children with large WC or high WHtR had significantly greater chances of being at high metabolic and cardiovascular risk than normal-weight children with a low WC measurement or WHtR [144]. The Kiel Obesity Prevention Study revealed that WC as well as BMI are appropriate to estimate cardiovascular disease risk factors but more detailed information may result from their combined use [149]. These findings along with those by Blüher, et al. [145] and Janssen, et al. [137] were not verified when using increased cIMT as outcome. The use of combined indices of total and central fat resulted in slightly lower prediction performance of cIMT than that achieved by WC alone. The strong association between BMI, TBFI, WHtR, WC and ABFI probably accounts for their similar predictive abilities, as well as for the absence of additional information obtained by combining measures.

LIMITATIONS

This was a cross-sectional study in a rather small sample of participants, thus no cause-effect can be established based on the present results. Due to the absence of specific exclusion criteria, sleep apnea, often associated with cardiovascular disease and atherogenesis may have passed unnoticed among the clinical characteristics of this population. However, Iannuzzi et al. [150] has demonstrated that obstructive sleep apnea was not associated with cIMT in children. The use of DXA for body composition assessment, albeit an objective instrument to assess body composition in a three compartment model, is unable to determine visceral adiposity independently from subcutaneous body fat. Nevertheless, previous studies indicate strong correlation between abdominal body fat estimated from selected range of interest assessed by DXA, and visceral body fat quantified by magnetic resonance imaging [151]. Although DXA technology is attractive in the pediatric population and regarded by many as a reference technique for body composition measurements, it is not without limitations. There is a continuing need for validation studies against accepted criterion methods in this population, particularly as new software or technological changes are introduced. We used age- and gender-specific WC cutoff points for youths developed in a nationally

representative sample. Using different reference databases and cut-offs would lead to differences in the association with cIMT.

There are no data per se indicating an increased risk of mortality in children with higher than normal cIMT, probably as a function of extremely low mortality rates due to atherosclerosis in children. Also, there is currently no longitudinal evidence tracking cIMT from childhood to adulthood, evaluating if high cIMT in childhood confers increased risk in adulthood. To evaluate early, subclinical disease, assessment of cIMT has been used extensively in children and young adults with known risk factors for cardiovascular disease. Increased cIMT relative to normal children has been demonstrated in pediatric patients with cardiovascular disease risk factors. In randomized clinical trials assessing statin therapy in children, diet, and exercise, cIMT was decreased in accordance with changes in other cardiovascular risk factors, whereas cIMT increased in the placebo groups, suggesting that cIMT is indeed a surrogate marker of atherosclerotic burden in children [35]. If cIMT changes throughout childhood, these changes are very small and we understand that their clinical or functional relevance may be questionable. We are also aware that these changes in cIMT are accompanied by increases in arterial size, including luminal diameter [52]. Therefore, it is uncertain whether some of the changes in cIMT that occur with age represent normal vascular adaptation or a pathological change.

CONCLUSION

Combination of total body fat assessment and central pattern of fat accumulation measures does not improve the prediction of increased cIMT in children. Simple surrogate measures of fatness can be used to predict increased cIMT urging for special attention to those children who exhibit an increased central body fat phenotype.

INSIGHTFUL DISCUSSIONS WITH PEERS

Has the equipment and software used for DXA been validated for adolescents?

This is a very relevant concern. DXA is now widely adopted for the measurement of the fat, fat-free soft tissue and bone mineral compartments of the body. The technique is attractive because it is non-invasive, is easily applied in pediatric population and the radiation dose is extremely small. Whereas it is regarded by many as a reference technique for such measurements, it is not without limitations. There is a continuing need both for inter-machine comparisons and validation studies against accepted criterion methods, particularly as new software or technological changes are introduced.

Considerable work has been published on the validation of earlier generation DXA instruments against suitable reference standards [152]. Such work is ongoing, mainly in terms of the application to populations not previously examined. A generally accepted reference standard is the 4-compartment model in which body fat is estimated from measurements of body density (by hydrodensitometry), total-body water (usually by deuterium dilution), and DXA bone mineral values. A large study conducted in 411 healthy subjects, aged 6 to 18 years compared percentage total body fat (TBF) measurements using a 4-compartment model with those using GE-Lunar DPX or DPX-L machines and pediatric software [153]. DXA underestimated %TBF in subjects with lower %TBF and overestimated it in those with higher %TBF. The relationship between the 2 methods was not affected by gender, age, ethnicity, pubertal stage, height, weight, or body mass index. Thus, the authors proposed that DXA has the capacity for clinical application including prediction of metabolic abnormalities associated with excess %TBF in pediatrics. Few validation studies have been reported using the more recently developed DXA machines. In 30 overweight and obese children, the Prodigy fan-beam densitometer (GE/Lunar, Madison, WI) significantly overestimated TBF in both males and females compared with a 4-compartment model [154]. These results were consistent with those in 141 9–17 year-old females, in whom a Hologic QDR-2000W (software version 5.56; Hologic, Inc, Waltham, MA) in pencil-beam mode overestimated the percentage of total body fat by 3.9% on average, compared with the four-compartment model, with 95% limits of agreement ± 6.7% [155].

Differences between machines continue to be a concern for whole-body and regional-body composition assessment. Thus, we understand that machines from different manufacturers tend to show greater differences than those from the same manufacturer. A comparison of the Hologic QDR-1000W pencil-beam with the 4500W fan-beam instruments in 13–18-year-old youths showed that at lower values of TBF and %TBF, the QDR-4500 gave higher measurements than the QDR-1000, whereas at higher values, this relationship was reversed. The QDR-1000 tended to give higher BMC measurements, with larger differences for higher values [156]. In addition, the variation in individual differences in fat-free soft tissue tended to be markedly greater at higher levels of fat-free soft tissue. These results supported earlier reports comparing Hologic pencil-beam (QDR-2000W or QDR-1000W) and fan-beam (QDR-4500A) instruments in children and adults [157, 158]. However, this concern is an issue for investigators upgrading their machines or embarking on multicenter studies which was not our case. There remains a need for more in vivo cross-calibration studies between scanners, including comparisons between the same models in different centers, thus allowing translational equations to be developed for data adjustment when necessary.

Carotid IMT is an early marker of atherosclerosis. Nevertheless, its changes can be observed within years. A more reliable tool is “endothelial function” evaluation. Please point out such a point and discuss it. The authors can take into account the paper from Miniello et al. [159].

Although the reviewer suggests that endothelial function evaluation is a more reliable tool, to our knowledge there is no evidence in pediatric literature supporting one surrogate marker of subclinical atherosclerosis in detriment of another.

The reviewer indicated the study of Miniello et al. [159] in which HOMA index was an independent predictor for brachial artery FMD worsening in a total of 150 children and adolescents not controlled for BMI. The results also outlined a non-statistical significance of the correlation between cIMT and HOMA index, suggesting that progressive higher HOMA index values did not influence the increase in carotid thickness but influenced vascular function. However, and as stated by the authors, these results in cIMT were not in agreement with literature in obese children and adolescents [96, 113, 160, 161]. Although providing interesting findings this study does not make a standard approach measure of

endothelial function such as measurement of flow–mediated dilation (FMD) a more reliable tool than cIMT.

In fact, FMD and associated changes in blood flow with ultrasound require very skill operators and expensive instrumentation, and acquiring reproducible results can be challenging [78]. For this reason, results may vary among research sites and application to clinical practice has been limited [162, 163]. In the last years, alternative methods that are less operator-dependent have been developed to measure endothelial function. These techniques utilize the same physiologic principles, and quantify reactive hyperemia as a change in fingertip perfusion pressure measured by tonometry [164-166] or temperature [167, 168] following brachial arterial occlusion. Both of these fingertip methods have been used to assess differences in clinical populations, including the Framingham Heart Study cohort [169, 170] as well as studies in children [165, 166, 171]. As for cIMT, approximately 70-80% of the measurement variability is due to differences among sonographers, which underscores the importance of an excellent imaging technique as well. In our study, all measures were performed by the same technician using an echo-tracking system indicating the standard deviation of 6 mean values, therefore increasing significantly the reliability of the measure. Although the measurement variability is considered to be generally small, error increases proportionally with increasing cIMT, suggesting that the usefulness of cIMT imaging may be even greatest in young and middle-aged individuals [79].

Although FMD measurements have been extensively performed in children to assess the effects of various conditions, there are relatively few data on normal developmental patterns of endothelial function compared to cIMT. The time course and peak FMD measured after occlusion has been inversely associated with arterial diameter and total cholesterol concentration, but not age [172]. Arterial diameter increases during childhood, indicating the possibility that changes in FMD may be more closely linked to growth and development than to age per se [78]. Another examination of determinants of FMD values in children and adults showed that FMD, measured as the percent change in brachial arterial diameter, was highest in children (10.7% FMD) and declined with increasing age in young adults (7.5%) and middle-aged/older adults (6.0%) [173]. However, adjusting FMD for shear stress eliminated the difference in FMD between children and young adults. Although the developmental changes of cIMT during the developmental years have not

been clearly elucidated too, some studies assessing the arterial wall dimensions and properties in a large population of healthy adolescents revealed significant variations with age and body size and markedly skewed distributions, which were in part related to concordant variations of body mass and blood pressure [52], but this finding has not been consistent, as others have found little change in cIMT during childhood [53]. cIMT increased linearly from 0.384 to 0.397 mm [52] and from 0.377 to 0.407 [1] between 10 and 18 years of age. Changes in cIMT are accompanied by increases in arterial size as well, including luminal diameter [52] suggesting that the increase in cIMT may be a function of increased overall arterial size. Nevertheless, postmortem studies characterizing the normal histological growth of the aorta in infants and children have shown that both intimal and medial thickness and density increase from birth throughout childhood [54].

Several external and patient-related factors can influence FMD results as compared to cIMT. Time of day [174, 175] and arterial size can influence FMD results. Brachial arteries smaller than 2.5 mm in diameter are difficult to measure [176]. Smaller arteries are associated with a greater percent FMD [177], and therefore, baseline size may be considered as a covariate in data analyses. Some researchers hypothesize that the increase in shear stress caused by flow through a smaller vessel results in greater nitric oxide release, thereby accounting for this observation [178]. Careful attention to detail is essential in reading brachial FMD studies.

Due to the absence of specific exclusion criteria, the clinical characteristics of the population should be better discussed. For example, Brunetti et al. [179] pointed out the role of OSAS in pediatric population and cardiovascular risk. Please discuss such a point.

A possible mechanism underlying an increased prevalence of atherogenesis among obstructive sleep apnea syndrome (OSAS) patients could be sympathetic activation [180], with consequent initiation and propagation of inflammatory responses within the microvasculature [150]. Also, it has become apparent that obesity and OSAS share many common pathways that lead to the induction of chronic inflammation that are now being elucidated in children. Up-regulation of pro-inflammatory signaling pathways particularly those mediated through NF-κB, HIF-1α, and metabolically active adipokines lead to increased expression of pro-atherogenic factors, thereby accelerating generation of

endothelial dysfunction and promoting the formation of lesions involved in atheromatous plaque formation [181, 182].

In an effort to identify pathophysiological links between obstructive sleep apnea-hypopnea and early markers of cardiovascular disease in the pediatric age group, Iannuzzi et al. [150] evaluated biochemical parameters and conducted carotid ultrasonography and polysomnography in a group of obese and lean children not selected for snoring. The authors demonstrated: (i) an association between the apnea-hypopnea index and high-sensitivity C-reactive protein concentrations, mainly mediated by overweight and obesity; (ii) a significantly greater low-grade inflammation in children with OSA than in children without OSA; and (iii) that OSA is not associated with subclinical atherosclerosis as measured by cIMT.

In adults, carotid markers of subclinical atherosclerosis are significantly higher in OSAS [183]. It is feasible that, in the pediatric age group, OSA, especially when associated with obesity, is able to elicit a low-grade inflammatory response, but, differently from adults [184, 185], it does not cause clear signs of carotid atherosclerosis although it may impair endothelial function as measured by flow-mediated dilation [179]. Thus, sleep disordered breathing and obesity may be part of a negative profile of cardiovascular risk that continues into adulthood, thereby facilitating development of cardiovascular morbidities.

6. INTIMA-MEDIA THICKNESS IN 11-13 YEARS-OLD CHILDREN: VARIATION ATTRIBUTED TO SEDENTARY BEHAVIOR, PHYSICAL ACTIVITY, CARDIORESPIRATORY FITNESS AND WAIST CIRCUMFERENCE

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ABSTRACT

BACKGROUND: It is unclear how sedentary behavior (SED), physical activity, and cardiorespiratory fitness (CRF) influence vascular structure in children of varying body size. This study examined whether associations between SED, physical activity and CRF with carotid intima-media thickness (cIMT) added to that of abdominal fatness and cIMT. Differences in physiological measures among waist circumference (WC) percentiles were tested.

METHODS: We assessed cIMT in 265 children aged 11-13 years-old (135 girls). Measures included cIMT assessed with high-resolution ultrasonography, WC, total body fat (TBF) from DXA and CRF determined using a maximal cycle test. SED and physical activity were assessed by accelerometry. Association between cIMT and CRF adjusted for physical activity variables, and body composition phenotypes were tested with multiple linear regression analysis.

RESULTS: CRF was related to cIMT independently of moderate to vigorous physical activity (MVPA) and SED ($p<0.05$). When WC was added to the model CRF was no longer associated with cIMT ($p>0.05$). Children in the higher WC group had increased mean values of BMI, TBF, WC and cIMT and lower MVPA and CRF ($p<0.05$).

CONCLUSION: Full modelling of SED, MVPA, CRF, and WC revealed that regional fat appears to have the biggest role in arterial structure of children.

KEYWORDS

Atherosclerosis, Children, Physical Activity, Cardiorespiratory Fitness, Waist Circumference, Intima-Media Thickness

INTRODUCTION

Children are not generally considered at risk for having clinical cardiovascular disease (CVD) events in the short term. However, the high prevalence of sedentary behavior (SED), low cardiorespiratory fitness (CRF) as well as clustering of cardio-metabolic risk factors during youth sets the stage for heart disease in adulthood [186, 187]. Recently, more specific arterial risk factors have been examined, including premature changes in carotid intima-media thickness (cIMT), an intermediate phenotype for early atherosclerosis, and an important predictor of future vascular events [133].

A systematic review of observational studies confirmed early vascular changes in obese and overweight pediatric populations with an increased future risk for vascular disease [97]. Moreover, increased intra-abdominal fat has been recently related to greater cIMT in children [94, 103, 119] showing that even moderate degrees of regional fat influence arterial structural and are related to cardiovascular risk [134].

Besides body composition phenotypes, the progression of atherosclerosis has been inversely associated with health-related variables such as physical activity and cardiorespiratory fitness (CRF) [66, 118]. However, the effect of CRF and physical activity on subclinical atherosclerosis measures in children reveals conflicting findings. Results from cross-sectional studies failed to establish CRF and physical activity as predictors of vascular structure in overweight youth [85] and type 1 diabetes mellitus children [188]. Similar findings were reported in a observational population-based study with adolescents [84]. Conversely, others have shown an inverse association of physical activity and IMT of the aortic artery in 13, 15 and 17 years-old adolescents [82] and a favorable effect of CRF on IMT of the aortic artery in 11 and 17-year-old adolescents [83]. Interventional studies in obese children have also shown that regular exercise is associated with improvements in cIMT leading to an improved cardiovascular risk profile [33, 63, 189, 190]. The source of this conflicting evidence has been partly attributed to differences in methodology, either

small or selected samples, use of self-reported physical activity or the use of instruments incapable to assess physical activity intensity [84]. In addition, none of the reported studies considered the role of SED on cIMT which may have deleterious effects on risk factors regardless of the degree of fatness in children [191].

Therefore, this study examined whether the association between SED, physical activity and CRF and cIMT in 11-13 years-old children is independent of the association between abdominal fatness and cIMT. Additionally, we tested for differences in physiological relevant measures among waist circumference (WC) percentiles.

METHODS

STUDY POPULATION

Participants were 265 children (135 girls) aged 11-13 years-old from 6 schools of the Lisbon district. The study was approved by the Faculty of Human Kinetics - University of Lisbon, Portugal. Informed consent was obtained from all subjects and their parents.

The study population was sequentially studied without specific exclusion criteria, hence the investigation did not specifically target children who were overweight/obese, or of any particular fitness level.

ANTHROPOMETRICS

All the anthropometric measurements were performed by trained observers. Height and sitting height were measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg on a scale with an attached stadiometer (model 770, Seca; Hamburg, Deutschland). Leg length was calculated by subtracting sitting height from height. Body mass index (BMI) was calculated as body mass divided by height squared ($kg.m^{-2}$). Waist circumference was measured to the nearest millimeter with an inelastic flexible metallic tape (Lufkin - W606PM, Vancouver, Canada) midway between the lower rib margin and the iliac crest, at the end of a gentle expiration. The mean value of the two closest measurements was used for analysis.

DUAL-ENERGY X-RAY ABSORPTIOMETRY

Total-body scans were performed by dual-energy radiographic absorptiometry (DXA) and analyzed using an extended analysis program for body composition (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, Waltham, Massachusetts, USA) to determine total body fat (TBF), used as an estimate of total body fatness. The same technician positioned the subjects, performed the scans and completed the scan analysis according to the operator's manual [192] using the standard analysis protocol. All scans were made in the morning after an overnight 12-hour fast. Quality control with spine phantom was made every morning, and with step phantom every week. Repeated measurements with DXA in 18 young adults showed a coefficient of variation of 1.7% for TBF.

MATURITY

Maturity offset, that is, time before or after peak height velocity (PHV), was predicted with the equation of Mirwald et al. [106]:

*Maturity Offset in boys = -9.236 + 0.0002708*Leg Length and Sitting Height interaction - 0.001663*age and leg Length interaction + 0.007216*age and sitting height interaction + 0.02292*weight by height ratio*

*Maturity offset in girls = -9.376 + 0.0001882*leg length and sitting height interaction + 0.0022*age and leg length interaction + 0.005841*age and sitting height interaction -0.002658*age and weight interaction + 0.07693*weight by height ratio.*

Length measurements were made in centimeters and weight measurements were in kilograms. The weight by height ratio was multiplied by 100.

PHYSICAL ACTIVITY

Physical activity was assessed by accelerometry (ActiGraph GT1M model; Fort Walton Beach, FL). All participants were asked to use an accelerometer on the right hip, near the iliac crest during four consecutive days, including two weekdays and two weekend days [193]. The delivery to and reception by the participants of the accelerometers, as well as

the explanation of its use, were personally made [194]. The devices were activated on the first day at 6.00 a.m., and data were recorded in 15-s epochs.

The device activation and data download were performed using the ActiLife Lifestyle software (v.3.2; Fort Walton Beach, FL). Processing was done using MAHUffe v.1.9.0.3 (available from www.mrc-epid. cam.ac.uk) from the original downloaded files (in DAT format).

For the analyses, a valid day was defined as having 600 min (10 h) or more of monitor wear, corresponding to the minimum daily use of the accelerometer [194]. Apart from accelerometer non-wear time (i.e., when it was removed for sleeping or water activities), periods of at least 60 consecutive minutes of zero activity intensity counts was also considered non-wear time. The study included the results from participants with at least three valid days (including one weekend day), with at least 10 h of wear time per day.

The amount of activity assessed by accelerometry was expressed in number of steps per day (STEPS) and as minutes per day spent in different intensities. The cut-off values used to define the intensity of physical activity and therefore to quantify the mean time at each different intensity were the following: moderate intensity 4-6 METs and vigorous intensity ≥ 7 METs. To adjust for the higher level of resting energy expenditure in younger people we determined the age-specific cut-off values according to the equation proposed by Trost et al. [195].

To analyze adherence to physical activity recommendations for public health, we considered the accumulation of at least 60 min of physical activity of moderate or greater intensity per day.

A SED cut-point of 100 counts·min^{-1} was used [196, 197].

CARDIORESPIRATORY FITNESS

Cardiorespiratory fitness (CRF) was determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer (Monark 828 E Ergomedic; Monark, Vansbro, Sweden). Initial and incremental workloads were 20 W for children weighing less than 30 kg and 25 W for children weighing 30 kg or more [198]. The

workload was increased every 3 minutes until the maximal effort of the participants was reached. Heart rate (HR) was collected continuously (Polar Vantage, Kempele, Finland) throughout the test.

Criteria defined for a maximal effort were HR >185 beats.min^{-1}, failure to maintain a pedaling frequency of at least 30 revolutions and a subjective judgment by the observer that the participant could no longer continue, even after encouragement [199]. The cycle ergometer was electronically calibrated once every test day and mechanically calibrated after being moved.

Maximal power output was calculated according to the following formula [200]: Power output = W1 + (W2t/180) where W1 = workload at the fully completed stage, W2 = workload increment at the final incomplete stage, and t = time (seconds) at the final incomplete stage. The test has been previously validated against direct measurement of VO_2max in 42 children aged 9 and 262 adolescents [198]. Correlations between maximal power output and VO_2max were 0.89 in both the 9 year olds and adolescents. The Hansen [200] formula for calculating CRF was:

VO_2max (ml.min^{-1}) = *12*calculated power output +5*body weight (in kg).*

For the analysis, CRF normalised by weight (ml.min^{-1}.kg^{-1}) was used.

HEMODYNAMICS

The HR at rest, brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 10 min with the participants in the supine position using an automated oscillometric cuff (HEM-907-E, Omron, Tokyo, Japan). Two measurements were taken and if these values deviated by >5 mmHg, a third measurement was performed. The average of the closest 2 values was used. The pulse pressure (PP) was calculated for adjustment purposes since PP was positively correlated with the mean cIMT of both the common and internal carotid arteries in a total of 128 Greek children and adolescents aged 10-19 years-old [201].

INTIMA-MEDIA THICKNESS

The IMT of the common carotid artery was defined as the distance between the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall of the carotid artery. Carotid ultrasound was performed on the right carotid artery using an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Genova, Italy) and implemented with a previously validated radiofrequency-based tracking of arterial wall that allows a real-time determination of common carotid far-wall thickness (QIMT®) with high spatial and temporal resolution [88].

Far-wall cIMT was automatically measured, and distension curves were acquired within a CCA segment ~1 cm before the flow divider, where the operator places the region of interest. From the distension curves, maximum and minimum carotid diameters are obtained. The coefficient of variation was 4.05% for cIMT and 2.71% for diameter.

STATISTICAL ANALYSES

All values were expressed as mean and S.D. Multiple linear regression analysis was used to estimate the association between the exposure variable (cIMT) and physical activity measures and CRF. We tested the association between cIMT and SED and adjusted the model for physical activity intensity (2^{nd} model), CRF (3^{rd} model) and body composition phenotypes (4^{th} model). Beta's and *P* were calculated for all models considering age, sex, maturity and PP as cofounders by including these variables as covariates in the regression model. Built on the results of the 4^{th} model, we categorized subjects according to smoothed sex- and age-specific percentile curve of WC for the Portuguese youth [109] [Lower WC (LWC): ≤50th; Middle WC (MWC): >50th-<85th; Higher WC (HWC): ≥85th]. Differences in SED, physical activity, CRF between the groups were assessed with ANOVA. Bonferroni test was used for post hoc comparison of means between each pair of groups. The statistical significance level was $p < 0.05$.

RESULTS

Prevalence of overweight and obesity in this study was 29.4% according to the cut-offs for normal weight, overweight and obesity proposed by Cole & Lobstein [108]. Taking into consideration the recommended levels of physical activity for children aged 5-17 years of the World Health Organization, 60.4% of the children in this study did not fulfill the recommended guidelines of 60 $min.d^{-1}$ of MVPA, although 60.4% of the entire cohort attained the recommended CRF level for metabolic health according to the cut-offs of Adegboye et al. [199] (TABLE 6)

TABLE 6: MEANS (± SD) OF THE CHARACTERISTICS OF THE STUDY GROUP AND DIFFERENCES BETWEEN WC PERCENTILE GROUPS.

		LWC	MWC	HWC	All
n		144	89	32	265
Age	(years)	11.42 ± 0.54	11.28 ± 0.47	11.25 ± 0.44	11.35 ± 0.51
Weight	(Kg)	39.00 ± 5.97	49.06 ± 5.54	61.56 ± 9.57*†	45.10 ± 9.93
Height	(cm)	149.21 ± 7.25	153.57 ± 6.60	154.39 ± 5.82*	151.30 ± 7.23
BMI	($kg.m^{-2}$)	17.43 ± 1.69	20.78 ± 1.85	25.78 ± 3.45*†	19.56 ± 3.43
WC	(cm)	62.03 ± 3.23	69.87 ± 2.74	81.47 ± 6.12*†	67.01 ± 7.36
TBF	(Kg)	9.27 ± 2.76	14.43 ± 4.00	24.14 ± 6.14*†	12.89 ± 6.14
Maturity	(years)	-1.14 ± 1.04	-0.67 ± 0.97	-0.35 ± 0.85	-0.89 ± 1.03
MVPA	(min)	58.73 ± 25.76	53.88 ± 22.33	42.65 ± 18.65*	55.13 ± 24.37
SED	(min)	538.50 ± 70.94	525.74 ± 70.18	558.46 ± 78.98	536.62 ± 72.11
Power output	($Watts.kg^{-1}$)	2.93 ± 0.52	2.51 ± 0.50	2.01 ± 0.39*†	2.68 ± 0.59
Rest HR	(bpm)	88.45 ± 14.50	88.12 ± 13.86	94.60 ± 14.83	89.09 ± 14.43
Max HR	(bpm)	195.70 ± 9.02	195.16 ± 10.51	197.31 ± 9.52	195.71 ± 9.59
CRF	($ml.kg^{-1}.min^{-1}$)	46.68 ± 7.03	39.14 ± 6.49	31.82 ± 5.18*†	42.35 ± 8.43
SBP	(mmHg)	108.19 ± 9.47	114.12 ± 10.64	118.87 ± 8.24*†	111.48 ± 10.45
DBP	(mmHg)	61.10 ± 7.41	63.08 ± 9.05	64.75 ± 6.95	62.21 ± 8.03
Diameter	(mm)	6.24 ± 0.46	6.33 ± 0.47	6.47 ± 0.44*	6.30 ± 0.46
cIMT	(mm)	0.48 ± 0.72	0.49 ± 0.08	0.53 ± 0.08*	0.49 ± 0.07

* Significant differences from LWC ($p<0.05$); † Significant differences from MWC ($p<0.05$)

The determinants of cIMT were examined in multivariate regression analyses in the entire cohort (TABLE 7). SED and MVPA were not associated with cIMT (1st model and 2nd model, respectively). CRF was associated with cIMT (3rd model) independent of age, sex and maturity. However, when body composition phenotype variables were added to the 3rd model, BMI as an estimate of total body fatness and WC as an estimate of the subcutaneous and intra-abdominal fat tissue in the abdominal region, CRF was no longer significantly associated with cIMT. In model 4 only WC was significantly associated with cIMT even when controlling for PP.

Subjects were categorized into WC tertiles. The highest relative prevalence of overweight and obese children (in relation to the total number of participants in each tertile) was found in the HWC group (96.9%) and the lowest in the LWC group (1.4%) (FIGURE 7). The highest relative prevalence of subjects who did not fulfill the physical activity recommendations for public health was found in the HWC group (78.1%) and the lowest in the LWC group (56.3%) (FIGURE 8).

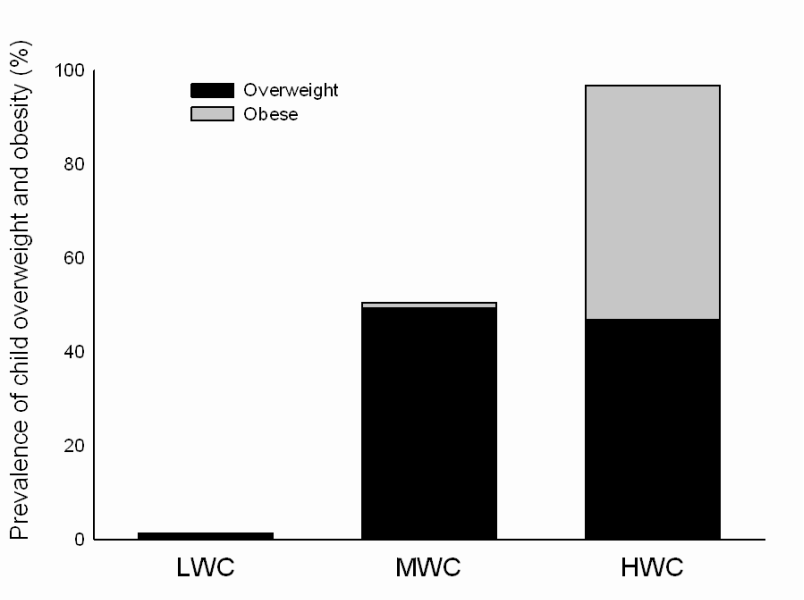

FIGURE 7: PREVALENCE OF CHILD OVERWEIGHT AND OBESITY BY WC PERCENTILE GROUPS IN THIS STUDY ACCORDING TO THE COLE & LOBSTEIN (2012) CRITERIA FOR BMI AND THE WC PERCENTILS BY SARDINHA ET AL. [.

A similar pattern was established in respect to the recommended CRF for metabolic health. The HWC group had the highest prevalence of children with CRF below the optimal cut-off values for identifying children with compromised metabolic health (90.6%). The LWC group had the highest prevalence of children (84.0%) above the optimal cut-off for CRF (FIGURE 9).

The three WC groups were similar for age, maturity offset, SED, DBP, HR at rest and maximal HR ($p>0.05$) (TABLE 6). As compared to LWC group, subjects in the HWC group had higher mean values of weight, height, BMI, WC, TBF, SBP, diameter and cIMT, and lower MVPA, power output and CRF ($p<0.05$). The subjects in the HWC group also had higher weight, BMI, WC, TBF and lower power output, CRF in comparison to the MWC group ($p<0.05$).

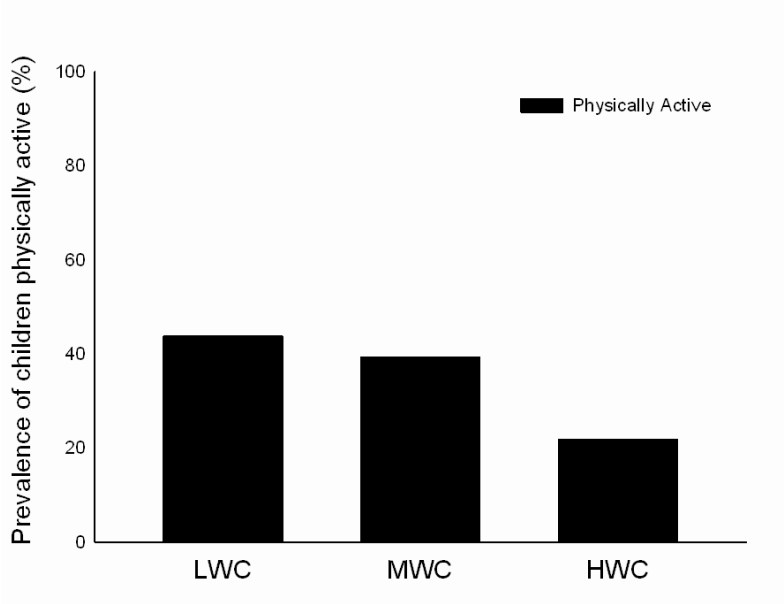

FIGURE 8: PREVALENCE OF PHYSICALLY ACTIVE CHILDREN BY WC PERCENTILE GROUPS, ACCORDING TO THE WC PERCENTILS BY SARDINHA ET AL. [109] AND THE RECOMMENDED LEVELS OF PHYSICAL ACTIVITY FOR CHILDREN AGED 5 - 17 YEARS - WORLD HEALTH ORGANIZATION.

DISCUSSION

We found that fat tissue in the abdominal region is associated with the arterial structure of 11-13 years-old children, whereas SED, MVPA, and CRF were not independently associated with cIMT after accounting for WC. Children with higher WC have increased cIMT, overall body fat and blood pressure, together with diminished physical activity and CRF. In addition, our study provides a novel finding showing that SED was not associated with cIMT independently of WC in 11-13 year old children.

TABLE 7: MULTIPLE REGRESSION ANALYSIS WITH cIMT AS DEPENDENT VARIABLE AND CRF, PHYSICAL ACTIVITY INTENSITY AND BODY COMPOSITION PHENOTYPES AS DETERMINANTS.

cIMT	Variables	Beta	p
Model 1	SED	-0.09	0.13
Model 2	SED	-0.09	0.13
	MVPA	-0.02	0.74
Model 3	SED	-0.08	0.17
	MVPA	0.04	0.57
	CRF	-0.13*	0.04
Model 4	SED	-0.09	0.99
	MVPA	0.03	0.95
	CRF	0.01	0.88
	BMI	-0.06	0.19
	WC	0.19*	0.002

The models 1-3 are adjusted for age, sex and maturity. Model 4 was additionally adjusted for PP; * Variables that entered the model

The prevalence of overweight and obesity in this study was 29.4% according to the cut-offs for normal weight, overweight and obesity provided by Cole & Lobstein [108]. This was quite similar to the 26.7% for this age range from the work of Sardinha et al. [110] in a representative sample of 10-18 years-old Portuguese youth. The highest relative prevalence of overweight and obese children was found in the HWC group in agreement with the evidence that WC and BMI are highly correlated ($r=0.90$; $p<0.001$; post-hoc analysis) even though they seem to measure different aspects of obesity [115]. For given

values of BMI, WC probably reflects abdominal fat deposits and for given WC, BMI probably reflects not only fat-free mass, but also fat deposits elsewhere in the obese BMI range.

The prevalence of physical inactivity was 60.4% taking into consideration the recommended levels of physical activity for children aged 5-17 years of the WHO. This was similar to the 63.6% reported in the study of Baptista et al. [202] for 10-11 year old children. This finding has potential public health implications, as it is known from cross-sectional and longitudinal observational studies that youth of both sexes who engage in relatively high levels of physical activity have reduced amount of fat than less active youth [203-208]. Our results also show this trend as children in the LWC group spent 16.08 $min.day^{-1}$ more in MVPA than children in the HWC group ($p<0.05$). Furthermore, post-hoc analysis showed a correlation of -0.24 ($p<0.001$; $n=265$) between WC and MVPA.

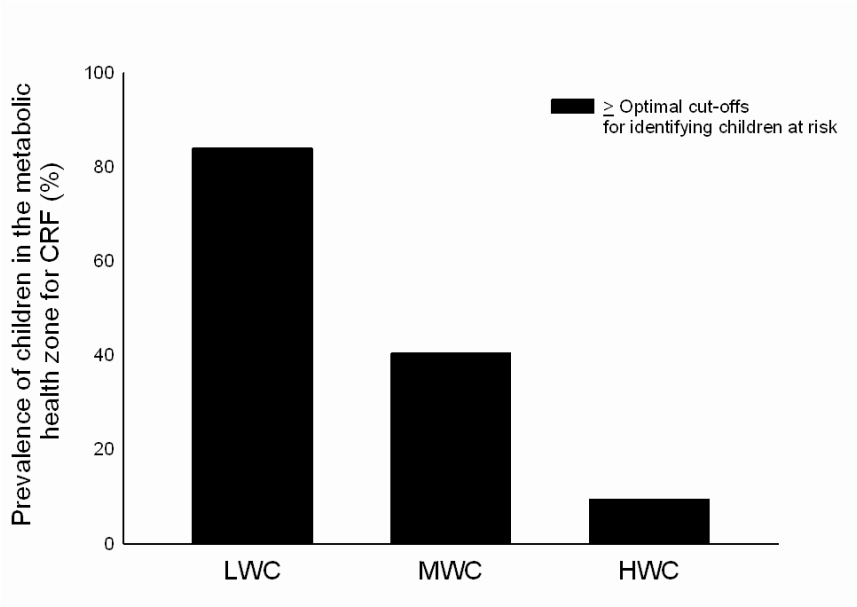

FIGURE 9: PREVALENCE OF CHILDREN ATTAINING THE RECOMMENDED CRF LEVEL FOR METABOLIC HEALTH ACCORDING TO THE WC PERCENTILS BY SARDINHA ET AL. [AND THE CRF LEVEL CUT-OFFS FOR CHILDREN AGED 9 AND 15 YEARS-OLD [199].

The group with higher subcutaneous and intra-abdominal fat tissue in the abdominal region (HWC) had the larger prevalence of children at risk of compromised metabolic health according to CRF cut-offs for 9 to 15 years-old children with CVD risk factors [199]. Anderssen et al. [209] reported a strong association between the different levels of CRF and the clustering of CVD risk factors in 2845 randomly selected 9-15 year old school children from Portugal, Denmark and Estonia. Odds ratios for the clustering of CVD risk factors between quartiles of CRF by country were higher in the Portuguese children in the lowest CRF quartile (17.3) after adjusting for maturity level. The mean CRF of the children in this cohort was lower than that reported in the study of Adegboye et al. [199] aimed at defining the optimal cut-off for low CRF and to evaluate its accuracy to predict clustering of risk factors for CVD in children and adolescents (42.35 ± 8.43 vs. 48.0 ± 8.7 $ml.kg^{-1}.min^{-1}$, respectively).

In a life-course approach, Ferreira et al. [210] found that not only current but also adolescent levels of CRF were inversely and independently associated with cIMT, but only in men (adjusted model for body height and body weight: β=-0.38, p=0.01). These results were extended by Pahkala et al. [83] reporting that in adolescents who were fit at age 17, the increase in aortic IMT and Young´s elastic modulus during the following 6 years was smaller compared with those who had the lowest CRF level. These associations suggest that CRF at an early age can influence carotid wall thickening, with important implications not only for understanding pathophysiological mechanisms, but also for public health policies. Our data on children support the findings from this and other studies in older subjects showing that high CRF is inversely associated with carotid atherosclerosis [66, 211]. However the association was no longer significant when this model was adjusted to subcutaneous and intra-abdominal fat tissue in the abdominal region. Thus, our data are consistent with various studies establishing a relation between central fat accumulation and the development and progression of carotid atherosclerosis [116, 117, 212, 213].

The differences between groups in fat accumulation, CRF, physical activity, and BP translates to physiologically relevant differences increasing both metabolic and cardiovascular risk. Exercise training may reduce cardiovascular events, which is likely at least partly mediated through the direct effects on vascular function and structure [214]. However, interventional studies have provided mixed results on the ability of exercise training to reverse vascular dysfunction and structure in children. Seeger et al. [215] for

example, showed that an 18-week exercise training program with predominantly running exercise in children with type 1 diabetes mellitus (10.9 ± 1.5 years) improved flow-mediated dilation (FMD) without altering cIMT or WC. Woo et al. [63] and Poeta et al. [190] showed that dietary and/or exercise intervention programs in overweight and obese 8-12 years-old children, produced only small changes in cIMT despite changes in %TBF and improvement of the metabolic profile. Meyer et al. [33] also concluded that regular exercise over 6 months restores endothelial function and improves cIMT associated with an improved cardiovascular risk profile in obese 14.7±2.2 years-old children. Although the main predictors of improved FMD seemed to be a percentage reduction in body weight, TBF, WHR, fasting insulin and increased physical activity, the same postulate has not been tested for the predictors of the reduction in cIMT. Nevertheless, the larger cIMT by 0.05 mm (50% of 0.1 mm) in our HWC group compared with LWC group in this study may result in a higher risk of CVD later in life, highlighting the importance of preventing obesity.

We did not find significant associations between the physical activity measures or CRF and cIMT within WC groups ($p>0.05$). Ried-Larsen et al. [84] reported similar results in 336 Danish adolescents (15.6 ± 0.4 years-old). Trigona et al. [188] also reported similar results in 32 patients with type 1 diabetes mellitus and 42 healthy subjects aged 6-17 years-old. In the healthy subjects group from their study, total physical activity accounted for 49% and MVPA for 34.3% of the variance of endothelial function, but not for cIMT. This seems to point out that changes in physical activity, CRF, TBF, cIMT and even FMD do not always coincide, thus the interaction of these variables has been difficult to ascertain, especially in children.

There are several possibilities why we did not observe an association of CRF and physical activity with cIMT after the adjustment for WC. First, given the young age of our sample, it is likely that our subjects had not been sufficiently exposed to alter structural measures. Although in children the effect of physical activity on IMT was reported to be similar at 3 different ages (13, 15 and 17 years-old) [82], Stensland-Bugge et al. [118] concluded that age was a modulator of the inverse relationship between physical activity and carotid atherosclerosis and the protective effects of physical activity were more pronounced at higher age (i.e. 60–69 and >70 years). The increasing effect of physical activity with age is also supported by findings from the Framingham Heart Study [216], in which a stronger protective effect of physical activity on stroke risk in older than in younger men was found.

Secondly, increasing CRF in order to reduce cIMT might be effective only in high-risk individuals [84]. Thirdly, part of the source of this conflicting evidence may be due to the use of subjective methods to assess physical activity and leisure time physical activity as in the study of Pahkala et al. [82]. Studies among children and adolescents using self-reported physical activity hold an extra limitation as child may be less able to recall their physical activity than an adult [217]. Fourthly, the artery measured may originate different results. For example, although Pahkala et al. [83] concluded that CRF was favorably associated with aortic IMT and elasticity, no association of CRF with the respective carotid indices was found. Nevertheless, our results are novel adding to the current understanding the evidence that WC and cIMT are associated in children, but that CRF or physical activity do not influence this association.

STRENGTHS AND LIMITATIONS

This study has several strengths and limitations. Automated edge-detection on the basis of RF signal processing of B + M mode US imaging is probably the most accurate method do detect cIMT [88, 107, 218]. SED and physical activity were objectively measured, a methodological procedure that is critical for accurate measurements in children because they tend to under report sedentary and light activities when self-report methods are used [219, 220]. This is particularly relevant of children's sedentary and light activities, and further unplanned, play, or "lifestyle" activities that cannot be accurately assessed by self-report methods, and children tend to perform large amounts of such activities, characterized by numerous short bursts of activity lasting mere seconds, interspersed with similar periods of recovery [221]. The gold-standard measure of CRF in humans involves direct assessment of VO_2peak or VO_2max in response to an exercise test. In this study CRF was indirectly determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer. However, CRF assessment protocol in the present study has been validated in children [198]. This was a cross-sectional study, thus no cause-effect can be inferred nor confirm the speculations made. The tertiles set by the authors may not predict risk of atherosclerosis, but as far as we know there is no considerable normative data in this age group. Even though reports postulate that cIMT is related to an initial atherosclerotic process [89, 222], at lower degrees of cIMT the thickening of the arterial wall may reflect an adaptive response to changes in shear stress,

lumen diameter, tensile stress, and pressure instead of an atherosclerotic thickening [223]. Regardless of whether cIMT reflects local atherosclerosis, it may serve as a graded marker for cardiovascular risk. Likewise, the ultrasound method for cIMT does not allow for differentiation between cIMT due to atherosclerotic process or medial hypertrophy (smooth muscle growth) due to pressure effects [224], although the authors recognize that cIMT may be more closely related to intimal atherosclerotic process.

CONCLUSION

In this sample of 11-13 years-old children, regional fat is a stronger predictor of arterial structure than SED, MVPA and CRF. Although CRF was associated with cIMT in a model including SED and MVPA, this association was no longer significant in a further model comprising WC. Higher WC is associated with higher cIMT, higher overall body fat and lower physical activity levels.

INSIGHTFUL DISCUSSIONS WITH PEERS

It is stated in the manuscript that regular exercise improves cIMT in obese individuals, but can you explain if this was related to weight loss (WC, body fat%, etc.)?

Accumulation of fat mass, in particular in the abdominal area, increases the risk of metabolic complications such as diabetes, dyslipidemia, hypertension and atherosclerosis, which are associated with a high morbidity and mortality [115]. A relation between central fat accumulation and the development and progression of carotid atherosclerosis has been established [116, 117] and concluded that atherosclerotic events could be avoided by preventing abdominal obesity. In a cross-sectional study our group showed a significant correlation between cIMT and WC in 11-13 years-old children, although this correlation was not significantly different from the correlation obtained between cIMT and BMI, body fat mass and trunk fat mass by DXA [134]. Interventional studies in obese children have in fact shown that regular exercise is associated with improvements in cIMT but provided limited information on the associations of changes in cIMT in relation to changes in other risk factors. Farpour-Lambert et al., [189] and Poeta et al. [190] for example stated that the intervention groups showed significant reductions in BMI, total cholesterol, LDL-

cholesterol, and DBP and even CRF after the end of the program but did not test if changes in cIMT were associated with changes in those variables. The study of Woo et al. [63] is also worth mentioning as the authors found that by the end of the intervention there was significant regression of cIMT in the children continuing exercise training with significant improvements in body fat content in children associated with significant changes in LDL cholesterol and HDL cholesterol but not BMI. Meyer et al. [33] actually tested for the relative changes in risk parameters but only for changes in flow-mediated dilation (FMD), not cIMT. The authors concluded that risk parameter changes possibly relating to FMD were BMI, waist/hip ratio, body fat, fasting insulin, and hours of weekly sports activities. Due to the limited information on this topic and to the design of this study that could not address it properly we only linked regular exercise to improvements in cIMT leading to an improved cardiovascular risk profile based on existent scientific evidence.

You speak about reasons for differences and focus on age, but your comparisons are to subjects quite about older. Can you make comparisons to an older group than your subjects, but not as extreme? Did you consider other methodological differences other than age (the way in which CRF and physical activity were measured)?

Determinants of cIMT in cross-sectional studies have been increasingly studied in children, but to our knowledge there is no population-based longitudinal study examining the long-term effect of age, sex, blood pressure, serum lipids, BMI, and physical activity on cIMT in children, challenging conclusions on time-dependent expression of genes, a gene-environment interaction or familial aggregation of environmental factors. Pahkala et al. [82] partly addressed this lack of prospective-based information by testing the association of leisure-time physical activity with endothelial function and aortic IMT in adolescents (13, 15 and 17 years-old). The authors concluded that physical activity was favorably associated with endothelial function and IMT in adolescents and that the effect of physical activity on IMT was similar at the three different ages (13, 15 and 17 years-old). Although derived from a 5 year follow-up, the stable effect of physical activity along different ages collides with the proposition by Stensland-Bugge et al. [118] that different risk factors are active in different stages of atherogenesis. Given the trend towards a greater protective effect of physical activity among elderly men, the authors hypothesized that risk factors found to

have a stable effect across age could be important in initiating atherosclerosis, whereas risk factors with increasing effect with age may be more associated with the progression of atherosclerosis. In the discussion of the submitted manuscript we wanted precisely to point out the cross-sectional interpretation of our data, advocating for a most possible and greater protective effect of physical activity to be found later in life through population-based longitudinal studies.

We also recognize other methodological differences that could be source of this conflicting evidence: 1) small or selected samples [225-229]; 2) the use of self-reported physical activity [82, 230] as it is known that children may be less able to recall their physical activity than adults [217] and 3) the artery measured. For example, although Pahkala et al. [83] concluded that CRF was favorably associated with aortic IMT and elasticity, no association of CRF with the respective carotid indices was found.

7. LINKING CARDIORESPIRATORY FITNESS CLASSIFICATION CRITERIA TO SUBCLINICAL ATHEROSCLEROSIS IN CHILDREN

Melo, X., Santa-Clara, MH., Santos, DA., Pimenta, NM., Minderico, CS., Fernhall, B., Sardinha, LB. Applied Physiology, Nutrition, and Metabolism, 2015. Apr; **40**(4): p. 386-392. JCR Impact Factor: 2.225 (2013). JCR Rank: Q1 (Sports Science).

ABSTRACT

BACKGROUND: It is unclear if cardiorespiratory fitness (CRF) can be used as a screening tool for premature changes in carotid intima-media thickness (cIMT) in paediatric populations.

PURPOSE: The purpose of this cross-sectional study was three-fold: 1) to determine if CRF can be used to screen increased cIMT; 2) to determine an optimal CRF cut-off to predict increased cIMT; 3) to evaluate its ability to predict increased cIMT among children in comparison with existent CRF cut-offs.

METHODS: cIMT was assessed with high-resolution ultrasonography and CRF was determined using a maximal cycle test. Receiver operating characteristic (ROC) analyses were conducted in boys (n=211) and girls (n=202) aged 11–12 years-old to define the optimal sex-specific CRF cut-off to classify increased cIMT (≥75th percentile). Logistic regression was used to examine the association between the CRF cut-offs with the risk of having an increased cIMT.

RESULTS: The optimal CRF cut-offs to predict increased cIMT were 45.81 and 34.46 ml.kg^{-1}.min^{-1} for boys and girls, respectively. The odds-ratios (OR) for having increased cIMT among children who were unfit was up to 2.8 times the odds among those who were fit (95%CI: 1.40-5.53). Considering the existent CRF cut-offs, only those suggested by Adegboye et al. [199] and Boddy et al. [231] were significant in predicting increased cIMT.

CONCLUSION: CRF cut-offs (boys: ≤45.8; girls: ≤34.5 ml.kg^{-1}.min^{-1}) are associated with thickening of the arterial wall in 11-12 years-old children. Low CRF is an important cardiovascular risk factor in children and our data highlight the importance of obtaining an adequate CRF.

KEYWORDS

Aerobic Evaluation; Intima-Media Thickness; Common Carotid Artery; Recommended Values; Pediatric; Risk

INTRODUCTION

There are many simple anthropometric and physiological measures that may predict the onset and progression of cardiovascular and metabolic disease. Body mass index (BMI) and waist circumference (WC) are accepted measures for this purpose and are associated with carotid intima-media thickness (cIMT) in children [97], an intermediate phenotype for early atherosclerosis [83, 210] and a solid predictor of future vascular events [133]. Although an inverse association between cardiorespiratory fitness (CRF) and metabolic risk in children has been shown [99, 209], and adolescent levels of CRF were inversely and independently associated with premature changes in cIMT in adults [210], the use of CRF as a screening tool for early subclinical atherosclerosis is not established in pediatric populations. This is potentially important because by assessing CRF, simple risk stratification can be performed in the school setting, and a strategy for detecting individuals at risk in public health settings for potential further investigation can then be created [199].

The purpose of this study was three-fold: 1) to determine if CRF can be used to screen increased cIMT in children; 2) to determine an optimal CRF cut-off to predict increased cIMT in children; and 2) to evaluate its ability to predict increased cIMT among children in comparison with existent CRF cut-offs for cardiometabolic health.

METHODS

STUDY POPULATION

Participants were 413 children (202 girls) aged 11 to 12 years-old, enrolled in 2012 from 6 schools of Portugal. This cross-sectional study was approved by the ethics committee of the Faculty of Human Kinetics - University of Lisbon, Portugal. Children provided assent for their participation and informed consent was obtained from their parents or legal tutors. The study population was sequentially studied without specific exclusion criteria, hence the

investigation did not specifically target children who were overweight/obese, or of any particular fitness level.

ANTHROPOMETRICS

Height and sitting height were measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg on a scale with an attached stadiometer (model 770, Seca, Deutschland), wearing minimal clothing and no shoes. Leg length was calculated by subtracting sitting height from height. Body mass index (BMI) was calculated and categorized according to the established criteria [108].

DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA)

Total-body scans were performed by DXA and analysed using an extended analysis program for body composition (Hologic Explorer-W, fan-beam densitometer, software QDR 12.4, USA) to determine total body fat and trunk fat. The coefficients of variation for repeated measurements in our laboratory for total and regional DXA measurements are reported elsewhere [232].

MATURITY

Maturity offset, that is, time before or after peak height velocity, was predicted with the equation of Mirwald et al. [106] using the following variables: leg length, sitting height, age, weight, and height.

CARDIORESPIRATORY FITNESS

CRF was indirectly determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer (Monark 828 E Ergomedic; Monark, Sweden). Initial and incremental workloads were 20 W for children weighing <30 kg and 25 W for children ≥30 kg [198]. The workload was increased every 3-min until the peak effort of the participants was reached. Heart rate (HR) was recorded continuously (Polar Electro Oy, Finland) throughout the test. Criteria defined for a peak effort were HR >185 bpm or the subjective judgment by the observer that the participant could no longer continue, even after encouragement. Peak power output and peak oxygen consumption ($ml.min^{-1}$) were

calculated according to the formulas by Hansen et al. [200]. Peak oxygen consumption was normalized by weight ($ml.kg^{-1}.min^{-1}$) and termed CRF from here on. The test has been previously validated against direct measurement of peak oxygen consumption [198].

HEMODYNAMICS

The HR at rest, brachial SBP and DBP were measured after 10 min with the participants in the supine position using an automated oscillometric cuff (HEM-907-E, Omron, Japan). Two measurements were obtained and if these values deviated by >5 mmHg, a third measurement was performed. The average of the closest 2 values was used. The pulse pressure (PP) was calculated for adjustment purposes since PP was positively correlated with mean cIMT [201].

INTIMA-MEDIA THICKNESS

cIMT was defined as the distance between the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall of the right carotid artery using an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy) and implemented with a previously validated radiofrequency-based tracking of arterial wall that allows a real-time determination of common carotid far-wall thickness (QIMT®) with high spatial and temporal resolution [88]. cIMT was automatically measured, and distension curves were acquired within a segment of the carotid artery about 1 cm before the flow divider, where the operator places the region of interest. The coefficients of variation for repeated measurements in our laboratory for carotid IMT and diameter are reported elsewhere [233].

STATISTICAL ANALYSES

Data are presented as means and standard deviation (SD) unless stated otherwise. All variables were checked for normality. Receiver operating characteristic (ROC) analysis were used to determine the efficacy of CRF for correctly identifying children with increased cIMT for each sex group. Early subclinical atherosclerosis was defined as cIMT≥75^{th} percentile (P75) for age, and sex [125] as defined by Doyon et al. [1] in a study aimed to establish a cIMT international reference data set for the childhood and adolescence

period. The decision for the optimal cut-off was the cut-off value with the highest accuracy that maximized the sum of the sensitivity and specificity. Participants from this study were then classified as fit or unfit using the generated cut-offs and those listed in TABLE 8.

Student's t-test was used to assess the statistical significance of the difference in cIMT and other physiologically relevant variables among CRF groups. Differences were tested controlling for other covariates (e.g. age and maturity offset) using the Bonferroni adjustment. Chi-square tests were performed for the comparison of proportions expressed as a percentage.

Odds-ratio (OR) with 95% confidence intervals (95%CIs) for having increased cIMT were calculated via multiple logistic regression entering sex and age as confounding variables. The statistical analyses were computed and analyzed using the SPSS Statistics 19.0 and MedCalc 10.1.2.0.

TABLE 8: HEALTH-RELATED CUT-OFFS FOR CRF IN CHILDREN AND ADOLESCENTS

Author	Sex	Sample (*n*)	Age (years)	Cut-offs ($ml.Kg.^{-1}min^{-1}$)	Method
Bell et al. [234]	Boys	---	Adolescents	40.0	Expert judgement
	Girls	---	Adolescents	35.0	
The Cooper Institute for Aerobics Research [235][a]	Boys	NHANES data	11	37.3	Criterion referenced standards
			12	37.6	
	Girls		11	37.3	
			12	37.0	
Ruiz et al. [236]	Boys	429	9-10	42.1	ROC analysis
	Girls	444		37.0	
Lobelo et al. [237]	Boys	677	12-15	44.1	ROC analysis
	Girls	570		40.3	
Adegboye et al. [199]	Boys	1219	9	43.6	ROC analysis
	Girls	1181		37.4	
Boddy et al. [231]	Boys	8382	9-11	46.6	ROC analysis
	Girls	8237		41.9	

[a] Updated data released in 2010 (http://cooperinstitute.org/healthyfitnesszone)

RESULTS

The descriptive characteristics of the participants are displayed in TABLE 9. Boys had lower BMI, total body fat, trunk fat, maturity offset, chronotropic responses, CRF and DBP than girls ($p<0.05$). No significant differences were found for cIMT ($p>0.05$). Overall prevalence of overweight and obesity in this study was 28.8% (boys: 24.6%; girls: 33.1%).

The potential of CRF to discriminate increased cIMT was higher than would be expected by chance. The diagnostic accuracy of the ROC generated thresholds was 0.60 (95% CI 0.53-0.66; p=0.07) in boys and 0.55 (95% CI 0.48-0.62; p=0.43) in girls. Sex dependent ROC curves analyses with generated and existent cut points, plus sensitivity and specificity values are shown in TABLE 10. In both boys and girls, the highest sensitivity was found with the criteria by Boddy et al. [231] whereas the highest specificity was found with the criteria by Bell et al. [234] in boys and by the generated criteria in girls.

TABLE 9: CHARACTERISTICS OF THE STUDY GROUP AND SEX COMPARISONS.

		Boys			Girls			All		
n		211			202			413		
Age	(years)	11.37	±	0.48	11.33	±	0.47	11.35	±	0.48
Weight	(kg)	43.65	±	10.55	45.93	±	9.51*	44.76	±	10.11
Height	(cm)	150.30	±	7.39	152.00	±	7.01*	151.13	±	7.25
BMI	($kg.m^{-2}$)	19.15	±	3.48	19.77	±	3.34	19.46	±	3.43
TBF	(kg)	11.20	±	6.12	14.13	±	5.73*	12.72	±	6.09
TF	(kg)	4.05	±	2.86	5.47	±	2.79*	4.80	±	2.91
Maturity	(years)	-1.82	±	0.58	-0.03	±	0.55*	-0.95	±	1.06
Rest HR	(bpm)	87.68	±	14.02	90.75	±	14.90*	89.18	±	14.52
Peak HR	(bpm)	193.50	±	11.49	196.25	±	10.26*	194.85	±	10.98
Power output	($W.kg^{-1}$)	2.88	±	0.64	2.47	±	0.48*	2.68	±	0.60
CRF	($ml.kg^{-1}.min^{-1}$)	44.84	±	9.02	38.87	±	7.03*	42.41	±	8.48
SBP	(mmHg)	110.32	±	9.64	111.38	±	11.44	110.84	±	10.56
DBP	(mmHg)	60.34	±	7.30	62.83	±	8.86*	61.56	±	8.09
Diameter	(mm)	6.41	±	0.47	6.11	±	0.45*	6.26	±	0.48
cIMT	(mm)	0.50	±	0.08	0.49	±	0.08	0.50	±	0.08
cIMT<P75[b]	(%)	13.0			15.2			14.0		

P75[b]: 75[th] percentile by Doyon et al. [1]; * Significant differences between boys and girls ($p<0.05$)

FIGURE 10 displays the prevalence of children who miss the recommended CRF according to the cut-offs generated in this study and those from TABLE 8. Significant differences were found between the prevalence of unfit boys (Bell et al. [234], The Cooper Institute for Aerobics Research [235] and Ruiz et al. [236]) and girls (The Cooper Institute for Aerobics Research [235], Ruiz et al. [236], Adegboye et al. [199], Boddy et al. [231]) by the cut-offs generated in the present study and those determined by the cut-offs in TABLE 8 ($p<0.05$). The highest prevalence of unfit boys and girls resulted from the cut-offs by Boddy et al. [231] whereas the lowest prevalence was set by the cut-offs of The Cooper Institute for Aerobics Research [235] in boys and those of the present study in girls.

TABLE 10: RECEIVER OPERATING CHARACTERISTIC CURVES ANALYSIS WITH CIMT≥P75[b] AS CLASSIFICATION VARIABLE IN BOYS AND GIRLS ACCORDING TO DIFFERENT CUT-OFFS*

	Boys			Girls		
	CRF Cut-offs	Sensitivity	Specificity	CRF Cut-offs	Sensitivity	Specificity
Bell et al. [234]	40.0	30.2	81.3	35.0	25.0	76.9
The Cooper Institute for Aerobics Research [235][a]	37.3	22.9	81.2	37.3	36.9	69.2
	37.6	24.0	81.2	37.0	35.2	69.2
Ruiz et al. [236]	42.1	36.3	78.1	37.0	36.9	69.2
Lobelo et al. [237]	44.1	45.8	75.0	40.3	53.4	53.9
Adegboye et al. [199]	43.6	45.8	78.1	37.4	36.9	65.4
Boddy et al. [231]	46.6	57.0	65.6	41.9	63.6	42.3
Present Study	45.8	52.0	75.0	34.5	24.4	88.5

* Coordinates of the ROC curve for approximate criterion values (0.1); [a] Updated data released in 2010 (http://cooperinstitute.org/healthyfitnesszone); P75[b]: 75th percentile by Doyon et al. [1].

Unfit children had higher BMI, TBF, TF, SBP, DBP, diameter and lower CRF ($p<0.05$) (TABLE 11). Participants classed as unfit by the generated CRF cut-offs in this study and those by Bell et al. [234], Lobelo et al. [237], Adegboye et al. [199] and Boddy et al. [231] had also significantly increased cIMT independently of PP in comparison to those classed as fit.

The OR for having increased cIMT among children who were unfit was up to 2.8 times the odds among those who were fit (TABLE 12). In addition to the CRF cut-offs generated in the present study, only those suggested by Adegboye et al. [199] and Boddy et al. [231] were significant in predicting increased cIMT.

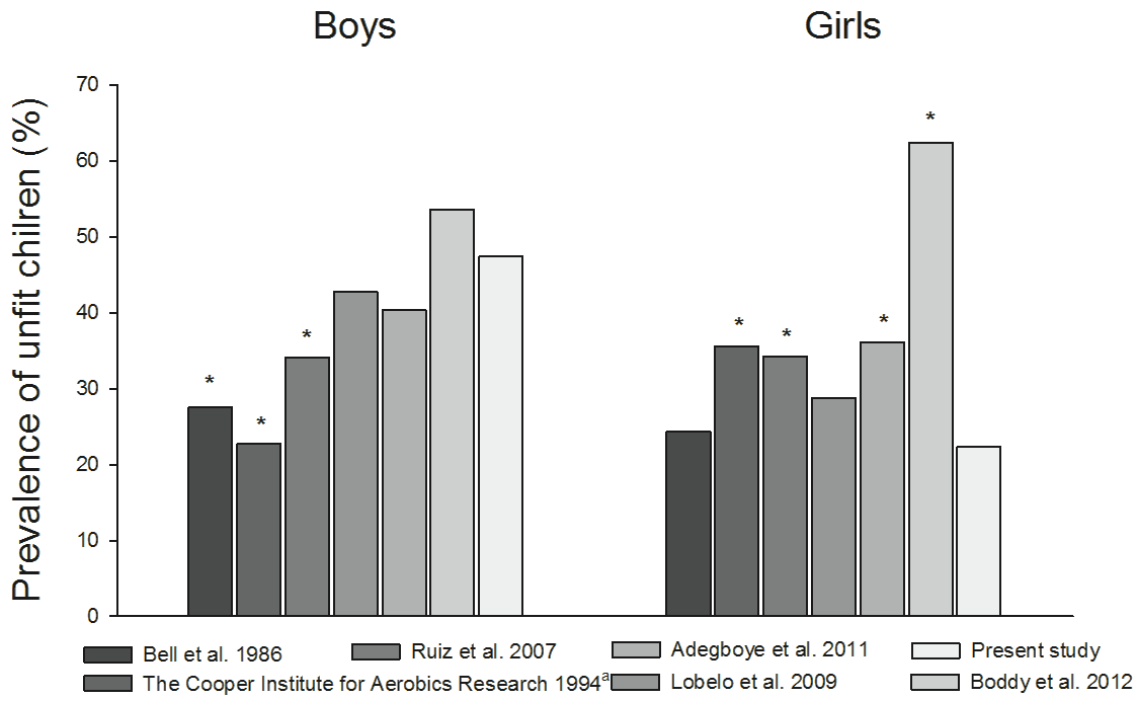

FIGURE 10: PREVALENCE OF CHILDREN ATTAINING THE RECOMMENDED CRF ACCORDING TO THE DIFFERENT CUT-OFFS FROM TABLE 8 AND THE GENERATED CRF STANDARDS FROM THE PRESENT STUDY.

[a] Updated data released in (2010); * Prevalence is significantly different from that of the present study using the generated cut-offs ($p<0.05$)

DISCUSSION

The results of the present study suggest that children failing to meet CRF standards have significantly increased cIMT compared to those who meet them. To our knowledge, this is the first study to show that low CRF in children is associated with a higher risk of increased cIMT while simultaneously strengthening the clinical validity of CRF thresholds for children in relation to their cardiovascular risk profile. Although there are no universally accepted recommendations for health-related levels of fitness in children, using the thresholds in TABLE 8 64.40±10.66% of boys and 61.11±13.27 of girls in the present study apparently have healthy CRF. These prevalence rates are lower than those reported for children in Australia (boys: 71.00%; girls: 77.00%) [238], USA (boys: 71.00%; girls: 69.00%) [237] and England (boys: 82.50%; girls: 84.15%) [239], and are in line with the prevalence rates for children from 10 European countries (boys: 61.00%; girls: 57.00%) [240]. The percentage of children failing to meet the required standards of CRF is of concern as associations exist between adolescent CRF and total body fat [241], serum lipids [242], subclinical atherosclerosis [210], large artery stiffness [243], and blood pressure [244], later in adulthood. Using cross-sectional designs, the present study and others [100, 209, 233] have also shown differences in physiologically relevant variables of body composition, hemodynamics and cIMT, according to CRF level in children. Several studies failed to establish CRF and physical activity as predictors of vascular structure in children and adolescents [84, 85, 188]. Conversely, others have shown an inverse association between physical activity and aortic IMT [82] and a favorable effect of CRF on aortic IMT but not cIMT in adolescents [83]. Interventional studies have also provided mixed results on the ability of exercise training to reverse subclinical atherosclerosis in children [33, 63, 215] so further longitudinal and/or intervention studies are needed to examine the impact of low CRF in childhood on the odds for atherosclerosis later in life.

TABLE 11: PHYSIOLOGICALLY RELEVANT VARIABLES ACCORDING TO CRF LEVEL

		Boys		Girls		All	
		Fit	Unfit	Fit	Unfit	Fit	Unfit
n		111	100	157	45	268	145
BMI	($kg.m^{-2}$)	17.21 ± 1.68	21.31 ± 3.70	18.75 ± 2.57	23.33 ± 3.23*	18.11 ± 2.36	21.94 ± 3.70*
TBF	(%)	20.77 ± 4.38	29.14 ± 7.46*	27.89 ± 5.67	36.71 ± 6.13*	25.17 ± 6.25	31.72 ± 7.88*
TF	(%)	16.22 ± 3.40	25.50 ± 8.60*	23.69 ± 6.83	33.99 ± 7.42*	20.83 ± 6.93	28.40 ± 9.13*
Rest HR	(bpm)	87.91 ± 13.34	87.44 ± 14.81	90.57 ± 15.00	91.37 ± 14.80	89.47 ± 14.35	88.66 ± 14.86
CRF	($ml.kg^{-1}.min^{-1}$)	51.66 ± 5.13	37.27 ± 5.79*	42.46 ± 5.55	30.81 ± 2.95*	46.27 ± 7.03	35.26 ± 5.89*
SBP	(mmHg)	108.27 ± 8.73	112.60 ± 10.13*	109.19 ± 10.17	119.02 ± 12.42*	108.81 ± 9.59	114.59 ± 11.25*
DBP	(mmHg)	58.90 ± 7.00	61.94 ± 7.30*	62.08 ± 8.19	65.44 ± 9.87*	60.76 ± 7.87	63.03 ± 8.31*
Diameter	(mm)	6.32 ± 0.48	6.51 ± 0.44*	6.07 ± 0.46	6.24 ± 0.38*	6.17 ± 0.48	6.42 ± 0.44*
cIMT	(mm)	0.49 ± 0.08	0.51 ± 0.08*	0.48 ± 0.08	0.52 ± 0.08*	0.48 ± 0.08	0.52 ± 0.08*
cIMT<P75[b]	(%)	21.6	8.0*	14.6	6.7	17.5	7.6*

Overall, no significant differences were found for age and maturity between groups of CRF level; * Significant differences between fit and unfit children ($p<0.05$); $P75^{b}$: 75^{th} percentile by Doyon et al. [1].

In terms of risk, children aged 9–10 years-old who met the FITNESSGRAM standards were 2-3 times (boys: 2.42; girls: 3.09) more likely to have a lower CVD risk score when compared to those who did not meet them [236]. Likewise, Lobelo et al. [237] found that boys aged 12-15 years-old who met the CRF standards were 5.17 times more likely to have a low CVD risk when compared to those who did not meet them, although statistical significance was not found for females. The results from our study extend these associations to children with early subclinical manifestations of cardiovascular pathology with important implications not only for understanding pathophysiological mechanisms, but also for public health policies. The risk for increased cIMT among children who were unfit was up to 2.8 times [1.82 Boddy et al. [231] and 1.92 Adegboye et al. [199]] the odds among those who were fit and the standards were significant in predicting increased cIMT.

The larger cIMT by 0.04 mm in the unfit group compared with the fit group in this study may result in a higher risk of CVD later in life. Schools may play an important role in identifying children with low CRF via standardized tests promoting positive fitness-enhancing behaviors [237]. Valuable CRF standards in the prediction of increased cIMT in children may be provided by Bell et al. [234], Lobelo et al. [237], Adegboye et al. [231], Boddy et al. [231] and those from the present study.

The prevalence of children in this study below the early subclinical atherosclerosis threshold set by Doyon et al. [1] is remarkably low. According to the author's international reference data set for children's cIMT, the P75 of 11-12 years old boys and girls varies between 0.405 mm and 0.413 mm. In our study, the P75 varies between 0.55-0.56 mm in boys and 0.53-0.55 mm in girls. These variances may partly be explained by differences in the site of measure and analysis technic.

TABLE 12: LOGISTIC REGRESSION ANALYSES ON ASSOCIATIONS BETWEEN CRF CUT-OFFS FOR CHILDREN AND INCREASED cIMT.

Authors	OR (sex adjusted)	Lower 95%CI	Higher 95% CI
Bell et al. [234]	1.60	0.80	3.21
The Cooper Institute for Aerobics Research [235][a]	1.50	0.81	2.76
Ruiz et al. [236]	1.58	0.84	2.95
Lobelo et al. [237]	1.79	0.95	3.37
Adegboye et al. [199]	1.92	1.03	3.58
Boddy et al. [231]	1.82	1.04	3.20
Present study	2.78	1.40	5.53

[a] Updated data released in 2010

The area under the curve values are similar [199, 237] or slightly lower than those of other studies [231, 245] aimed to define optimal cut-offs for CRF and to evaluate its ability to predict clustering of cardiovascular risk factors among children and adolescents. Unsurprisingly, cut-offs proposed by the previous works were relatively similar to those generated in this study in boys [199, 231, 237] and girls [234, 237]. Resemblances among the cut-offs truly support the existence of a theoretical health-related standard value for CRF in children linked to a more favorable CVD risk profile, even when accessed by a subclinical measure of atherosclerosis such as cIMT.

LIMITATIONS

This study did not have the ambition to create a standard classification system for CRF. Rather, the findings presented were intended to serve as a hypothetical basis and stimulus to consider international healthy ranges of CRF based on cIMT in a pediatric perspective. These cut-off values ought to be evaluated in a wider separate test group in whom outcomes could be verified independently, thereby allowing the definition of groups with and without increased cIMT.

CRF was indirectly determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer. A recognized limitation on the use of cycle ergometers in Portuguese children is that they are not used to cycling [99, 209]. Therefore,

the cycle ergometer test used may be less suitable for Portuguese children, and this might explain their lower CRF level and peak power output [99, 199]. However, we did use a maximal test requiring a peak effort, which is a better indicator of CRF than submaximal tests [246]. Some children did not reach the maximal HR threshold, but these children did not have a different cIMT. Thus this was unlikely to affect our findings. Although the method by Hansen et al. [200] was designed to provide gross peak oxygen consumption values, a high correlation was also demonstrated between the directly measured value and the calculated value relative to body weight (r=0.84-0.96), indicating that this method of predicting peak oxygen consumption could be used as an accurate and valid method of establishing CRF levels when scaled to body weight.

The sex adjusted areas under the curve for the generated cut-offs were not significant, mostly likely due to sample size as a strong trend towards significance was found in boys, an important step conferring utility of the prediction. Still, the observed area under the curve and the sensitivity, especially in girls, was low which may indicate a low discriminatory ability. However, sensitivity and specificity of a test may have different consequences depending on the clinical and public health settings of interest. In view of the benefits that regular aerobic exercise has in children, increasing CRF level [247] and reducing CVD risk [248], false-positive cases (failing to identify children as high risk who really are at high risk) are of greater concern than false-negative cases (increasing CRF level of low-risk children) [199].

CONCLUSION

CRF was associated with early subclinical atherosclerosis in 11-12 years-old children. In addition, results suggest a theoretical CRF cut-off (boys: ≤45.8; girls: ≤34.5 $ml.kg^{-1}.min^{-1}$) to predict increased cIMT in children. Low CRF is an important predictor of a potent cardiovascular risk factor in children and our data highlight the importance of obtaining an adequate CRF to refer unfit children onto intervention programs/services.

INSIGHTFUL DISCUSSIONS WITH PEERS

The Hansen method was designed to provide gross VO2 ($l.min^{-1}$) and standard physiological theory suggests we don't use $ml.kg^{-1}.min^{-1}$ to scale VO2 from non-weight bearing tests. Can you please justify?

This is a very pertinent point raised by the reviewer. Although the Hansen et al. [200] method was designed to provide gross VO2, a remarkable and extremely high correlation was demonstrated between the directly measured value of VO_2max and the calculated absolute or relative value (0.84 to 0.96). This high degree of correlation was also demonstrated in the regression analysis, where the average standard error of estimation for VO_2max (calculated) on VO_2max (measured) was 0.06 $l.min^{-1}$ and 1.6 $ml.kg^{-1}.min^{-1}$, respectively, which corresponded to a standard error of estimation of less than 3.2% in the mean values of measured VO_2max and indicated that this method of predicting VO_2max could be used as an accurate and valid method of establishing individual physical fitness levels even with a body mass scaling factor.

We agree with the reviewer as body mass may not adequately reflect the metabolically active skeletal muscle mass, not only due to the exercise mode used but also due to the heterogeneity of body composition [249]. However, VO_2max per kg in the present study is very well correlated with the duration of the exercise test ($r=0.82$; $p<0.05$). In addition, using Z-statistic to perform ROC curve comparisons [250], the potential of CRF to discriminate increased cIMT in girls did not differ when CRF was expressed relative to body weight or when expressed in absolute values ($p>0.05$), and absolute CRF had actually no discriminative potential of increased cIMT in boys (AUC = 0.48). Theoretically, scaling the data by fat free mass, in particular that of the exercising limbs would gather more agreements. However, the potential of CRF to discriminate increased cIMT, did not differ when CRF was expressed relative to fat free mass from that when CRF was expressed relative to body weight or when expressed in absolute values ($p>0.05$). In addition, scaling by fat free mass relies on the assumption that the involved metabolically active muscle-mass at VO_2max represents a constant proportion of fat free mass across the sample which is not the case in children [249]. What we might not account for when expressing CRF by body mass is the variability in biologic development in the study sample, which affects performance fitness [251]. It follows that VO_2max per kg cannot

serve as a marker of changes in endurance fitness over time in children, but that was also not the aim of the present study nor did the age range of the participants allow that. According to some [249], an estimate of a proportion of the involved musculature ($VO_2max/Vol^{0.64}$) is a more valid allometric scaling denominator than either body mass or fat free mass but mainly for partitioning out the influence of body size on VO_2max which again was not the case in the present study as the sample was reasonably homogeneous. So we believe that our results do not provide evidence to use CRF scaled differently than the comparison studies used in the manuscript [199, 231, 234-237], some of which also used maximal cycle ergometry to estimate CRF [199, 236].

The children achieved a pretty low power output compared with the reference population in Odense (probably due to the inclusion of lots of 'Scandinavian cyclists' in the latter sample) but this appears to be coupled with a very high VO2peak. Is this evidence of low efficiency in cycling in your sample? Or is it just a problem with the method? I don't think many Portuguese children cycle to school do they? A brief comment is required, but my guess is that you have overestimated VO2peak.

This is a very interesting point raised by the reviewer and has been subject of discussion before. Anderssen et al. [209] examined the association between fitness and clustered CVD risk in 2845 randomly selected 9-15 year old school children from Portugal, Denmark and Estonia, and reported a strong association between the different levels of CRF and the clustering of CVD risk factors. Odds ratios for the clustering of CVD risk factors between quartiles of CRF by country were higher in the Portuguese children in the lowest CRF quartile (17.3). One of the self-recognized limitations of their study was that Portuguese children were not used to cycling. Therefore, the cycle ergometer test used may be less suitable for Portuguese children, and this might explain why they showed the lowest CRF level compared with the other countries. The mean CRF of the children in the present study was actually lower than that reported in the study of Adegboye et al. [199] aimed at defining the optimal cut-off for low CRF determined during a maximal test on a cycle ergometer, and to evaluate its accuracy in predicting clustering of risk factors for CVD in children and adolescents (boys: 44.80 ± 9.00 vs. 50.50 ± 8.70 $ml.kg^{-1}.min^{-1}$ at the age of 9 or 48.20 ± 9.20 $ml.kg^{-1}.min^{-1}$ at the age of 15; girls: 38.90 ± 7.00 vs. 45.40 ± 8.20

$ml.kg^{-1}.min^{-1}$ at the age of 9 or 52.40 ± 8.20 $ml.kg^{-1}.min^{-1}$ at the age of 15). In addition, the peak power output attained in this study although greater both in boys (2.88 ± 0.64 vs. 2.54 ± 0.61 at the age of 9 or 3.26 ± 0.56 at the age of 15) and girls (2.47 ± 0.48 vs. 2.01 ± 0.47 at the age of 9 or 2.16 ± 0.37 at the age of 15) than that reported by Andersen et al. [99] for the Portuguese cohort (n=370) in their multi-center study, was remarkably lower than those of participants from Denmark (boys: 3.21 ± 0.55 at the age of 9 or 3.75 ± 0.59 at the age of 15; girls: 2.86 ± 0.51 at the age of 9 or 3.03 ± 0.47 at the age of 15) and Estonia (3.20 ± 0.48 at the age of 9 or 3.53 ± 0.54 at the age of 15; girls: 2.77 ± 0.56 vs. 2.54 ± 0.61 at the age of 9 or 2.60 ± 0.40 at the age of 15).

A quick look at normative values by Catley et al. [238] or Sandercock et al. [239] from the 20 m shuttle run suggests your VO_2 estimates are rather high. You have to convert median shuttle run to VO_2peak based on Leger's equation of similar unfortunately – but your children seem rather fit. This must be a product of the method used. I suggest creating a cut-points using L/min VO_2 and comparison to highlight the limitation of using ml/kg/min from a cycling protocol – hopefully you will get a bigger area under the curve using this method!

We understand the reviewer's concern but higher mean values of peak oxygen uptake were reported in the mentioned studies. The mean peak oxygen uptake in the present study was 44.84 $ml.kg^{-1}.min^{-1}$ in boys and 38.87 $ml.kg^{-1}.min^{-1}$ in girls whereas the mean values in the centile curves and normative values by Sandercock et al. [239] ranged from 45.30-45.90 $ml.kg^{-1}.min^{-1}$ in boys and 41.90-43.00 $ml.kg^{-1}.min^{-1}$ in girls. Compared to the normative health-related fitness values for children by Catley et al. [238] and using the equation by Leger et al. [252] to estimate peak oxygen uptake ($ml.kg^{-1}.min^{-1}$) from maximal speed attained ($km.h^{-1}$), boys in the present study had a mean peak oxygen uptake around P50 (42.86-44.57 $ml.kg^{-1}.min^{-1}$) whereas girls were around P30 or P40 (37.77-39.64 $ml.kg^{-1}.min^{-1}$). Physical fitness percentiles for Portuguese children and adolescents have also been recently published [253]. Compared to this national representative sample, peak oxygen uptake in boys in the present study was around P50 (44.57-45.40 $ml.kg^{-1}.min^{-1}$) and P25-P50 in girls (42.11-42.86 $ml.kg^{-1}.min^{-1}$).

Although there are no universally accepted recommendations for health-related levels of fitness in children, sex- and age-specific threshold values for cardiovascular fitness have

been established for children based on expert judgement [234], criterion reference standards or, more recently, using linked cardiometabolic risk-based values from receiver operator characteristic curve analyses [199, 231, 236, 237]. Using these thresholds, 64.40±10.66% of boys and 61.11±13.27% of girls in the present study apparently have healthy CRF. These prevalence rates are lower than those reported for children from Australia (boys: 71.00%; girls: 77.00%) [238], USA (boys: 71.00%; girls: 69.00%) [237] and England (boys: 82.50%; girls: 84.15%) [239], and are in line with the prevalence rates for children from 10 European countries (Austria, Belgium, France, Germany, Greece, Hungary, Italy, Spain and Sweden) (boys: 61.00%; girls: 57.00%) [240].

8. INDEPENDENT ASSOCIATION OF MUSCULAR STRENGTH AND CAROTID INTIMA-MEDIA THICKNESS IN CHILDREN

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ABSTRACT

PURPOSE: The aim of this cross-sectional study was to examine the influence of muscular strength on carotid intima-media thickness (cIMT) in children, controlling for the effect of cardiorespiratory fitness (CRF) and central fat and to examine if differences among muscular strength tertiles translate to physiological differences.

METHODS: We assessed cIMT of the common carotid artery in 366 children between 11-12 years of age (191 girls). Measures included cIMT assessed with high-resolution ultrasonography, a maximal handgrip strength test, body fat mass and lean mass from DXA and CRF determined using a maximal cycle ergometer test. Association between muscular strength and cIMT adjusted for CRF and central fat, as measured by trunk fat, was tested with multiple linear regression analysis. Differences in risk factors among muscular strength groups were tested with ANOVA.

RESULTS: The Muscular Strength Index (MSI) was inversely associated with cIMT independently of CRF and central fat ($p<0.05$). The low MSI group had the highest values of cIMT, waist circumference and systolic blood pressure and the lowest CRF ($p<0.05$).

CONCLUSION: There was an inverse and independent association between muscular strength and cIMT. Low muscular strength was associated with higher levels of cardiovascular disease risk factors in children.

KEYWORDS

Handgrip; Cardiorespiratory Fitness; Central Fat; Intima-Media Thickness

INTRODUCTION

High levels of cardiorespiratory fitness (CRF) are associated with reduced cardiovascular morbidity and mortality in adults [81, 254]. Although muscular strength has received less attention than CRF, low muscular strength in adulthood also predicts all-cause mortality, as well as mortality due to cardiovascular disease (CVD) [255, 256]. A large prospective study verified that high muscular strength in adolescence, as assessed by knee extension and handgrip tests, was associated with a 20-35% lower risk of premature mortality due to any cause or CVD independently of body mass index (BMI) or blood pressure later in life [257]. Thus, muscular strength appears to be an important marker of cardiovascular health throughout the lifespan.

Greater muscular strength in youth is also associated with lower levels of CVD risk factors [258], including fasting insulin, markers of insulin resistance and beta-cell function in young adulthood independently of CRF and fat [259]. These data suggest that muscular strength in youth is important for maintaining healthy insulin sensitivity and beta-cell function later in life. However, whether muscular strength is associated with more specific arterial risk factors with a focus on target-organ damage has not been tested to date. Alterations of the cardiovascular system can be identified at an early age and premature changes in carotid intima-media thickness (cIMT), an intermediate phenotype for early atherosclerosis, and an important predictor of future vascular events [133] have been increasingly examined. cIMT may be reduced with regular exercise in children [33] and an improved fitness level, in particular CRF, is inversely associated with cIMT in children, although central fat appears to be more related with cIMT than CRF [233]. However it remains unknown whether other physical fitness dimensions such as muscular strength may also be related to cIMT in children, independently of CRF and central fat. If there are health benefits of muscular strength early in life, independent of engagement in other physical activities, increasing muscular strength could be recognized as an important target for intervention. Therefore, the purpose of this study was to analyze the independent association of muscular strength with cIMT in 11-12 years-old children controlling for the effect of CRF and central fat. Additionally we examined whether differences among muscular strength tertiles translate to physiological differences in CVD risk.

METHODS

STUDY POPULATION

Participants were 366 children (191 girls) between 11 to 12 years of age, enrolled in 2012 from 6 schools of Portugal. The study was approved by the ethics committee of the Faculty of Human Kinetics - University of Lisbon, Portugal and performed in accordance with the ethical standards of the International Journal of Sports Medicine [260]. Children provided assent for their participation and informed consent was obtained from their parents or legal guardians. The study population was sequentially studied without specific exclusion criteria, hence the investigation did not specifically target children who were overweight/obese, or of any particular fitness level.

ANTHROPOMETRICS

Standing and sitting height were measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg on a scale with an attached stadiometer (model 770, Seca, Deutschland), wearing minimal clothing and no shoes. Leg length was calculated by subtracting sitting height from standing height. Body mass index (BMI) was calculated and categorized according to the established criteria [108]. Waist circumference (WC) was measured to the nearest millimeter with an inelastic flexible metallic tape (Lufkin, W606PM, Canada) midway between the lower rib margin and the iliac crest. Age- and gender-specific WC percentiles for Portuguese youths [109] were used to dichotomize WC as normal (<P85) and increased risk (≥P85).

DUAL-ENERGY X-RAY ABSORPTIOMETRY

A total-body scan was performed by dual-energy radiographic absorptiometry (DXA) and analyzed using an extended analysis program for body composition (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, USA) to determine total, trunk and upper limb body fat and lean soft tissue. Trunk fat was used as an estimate of a central pattern of fat (visceral + subcutaneous) distribution. The same technician positioned the subjects, performed the scans and completed the scan analysis according to the operator's manual using the standard analysis protocol. All scans were made in the

morning after an overnight 12-hour fast. The coefficients of variation for repeated measurements in our laboratory for total and regional DXA measurements have been reported elsewhere [232].

MATURITY

Maturity offset, that is, time before or after peak height velocity, was predicted with the equations of Mirwald et al. [106] using the following variables: leg length, sitting height, age, weight, and height.

MUSCULAR STRENGTH

Maximal isometric forearm grip strength was determined on the dominant arm using a handgrip dynamometer (Lafayette, Model 78010, Lafayette Instrument Company, USA). The test was performed in the standing position with the arms alongside the body, without contact with the trunk and with the elbow of the dominant arm slightly flexed (~20º). A standard instruction of 'push as hard as you can' and visual feedback of the recorded strength was provided to each participant in each trial. The maximum trial of two attempts (10s of contraction with a rest period of at least 60s) was used as peak absolute force (kg). A percentile 20 of the derived sex- and age-specific normative values for physical fitness [240] was used to define a "Very Poor Level" of muscular strength. Absolute muscle strength may be the simplest way to express muscle strength, especially when measured with weight-bearing tests, since it does not require other measurements. However, handgrip strength is positively associated with weight status [261] and as a result, relative muscle strength may be more relevant in understanding health-related fitness, especially when measured with a non-weight-bearing tests as in this study. The DXA method provides lean soft tissue estimates of the extremities, and a large proportion of total-body skeletal muscle is within the fat-free appendicular compartment [262]. We calculated a muscular strength index (MSI) dividing peak absolute force (kg) by the upper limbs' lean soft tissue (kg) [263].

CARDIORESPIRATORY FITNESS

CRF was indirectly determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer (Monark 828 E Ergomedic; Monark, Sweden). Initial and incremental workloads were 20W for children weighing <30kg and 25W for children ≥30kg [198]. The workload was increased every 3-min until the maximal effort of the participants was reached. Heart rate (HR) was recorded continuously (Polar Electro Oy, Finland) throughout the test. Criteria for maximal effort were HR >185 beats.min^{-1} and a subjective judgment by the observer that the participant could no longer continue, even after encouragement. Maximal power output and maximal oxygen consumption (ml.min^{-1}) were calculated according to the formulas by Hansen et al. [200]. Maximal oxygen consumption was normalized for body weight (ml.min^{-1}.kg^{-1}) and termed CRF from here on. The test has been previously validated against direct measurement of maximal oxygen consumption [198].

HEMODYNAMICS

Heart rate at rest, brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 10-min with the participants in the supine position using an automated oscillometric cuff (HEM-907-E, Omron, Tokyo, Japan). Two measurements were taken and if these values deviated by >5 mmHg, a third measurement was performed. The average of the closest 2 values was used. The pressure difference between the SBP and DBP (PP) was calculated for adjustment purposes since both were positively correlated with the mean IMT of both the common and internal carotid arteries in a total of 128 children and adolescents between 10-19 years of age [201].

INTIMA-MEDIA THICKNESS

cIMT was defined as the distance between the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall of the carotid artery (FIGURE 11). The far wall was imaged and cIMT and carotid diameter were measured in a common carotid artery segment ~1 cm before the bifurcation. The coefficients of variation for repeated measurements in our laboratory for cIMT and diameter are reported elsewhere [134].

CARDIOVASCULAR RISK SCORE

In 2010 the American Heart Association released a set of 7 cardiovascular health metrics for children and adults to describe ideal cardiovascular health [264]. The metrics used to indicate cardiovascular health included 4 health behaviors and 3 health factors. The behavioral criteria were nonsmoking, being physically active, having normal body mass index (BMI), and eating a healthy diet. Normal blood pressure, total cholesterol, and plasma glucose levels indicated ideal health factors. Ideal cardiovascular health was defined as having all 7 metrics of the behaviors and factors. In this study, using adapted metrics and criteria of individual cardiovascular health, we calculated an optimal cardiovascular health score mainly for behavioral criteria and health factors (TABLE 13) where a value of 0 was assigned for each metric in the presence of risk and a value of 1 if the optimal criterion was met. The range of scores was thus 0 to 5, with a higher score indicating a higher cardiovascular health profile.

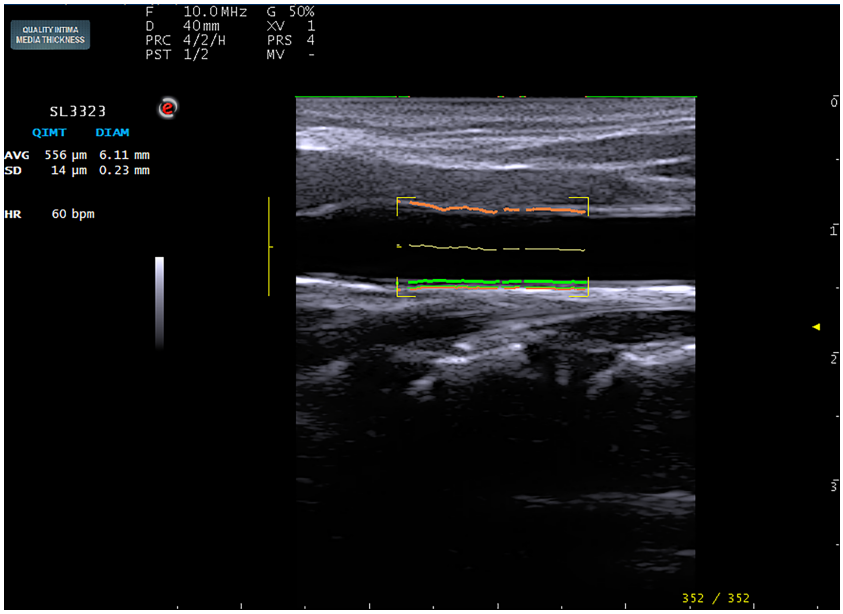

FIGURE 11: CAROTID ULTRASOUND IMAGE.

Carotid ultrasound was performed on the right carotid artery using an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy) using a validated radiofrequency-based tracking of arterial wall that allows a real-time determination of common carotid far-wall thickness (QIMT®) with high spatial and temporal resolution [88].

STATISTICAL ANALYSES

All values were expressed as mean and S.D. Multiple linear regression analysis was used to estimate the association between cIMT with physical fitness and body composition phenotypes. We tested the association between cIMT and MSI and then adjusted the model for CRF (2nd model), and central fat (3th model). R^2, Beta (standardized) and 95% CI were calculated for all models which were adjusted for sex, maturity offset and PP.

Subjects were categorized according to the number of optimal cardiovascular health metrics [Low Score (≤2); Middle Score (3-4) and High Score (5)] and MSI level [low MSI (LMSI): ≤7.23, middle MSI (MMSI): >7.23 and <8.23, and high MSI (HMSI): ≥8.23]. Physiological differences between groups and MSI level were assessed with one-way ANOVA and ANCOVA. LSD post hoc test was used for unadjusted and adjusted (diameter of the artery, sex, maturity, CRF, and trunk fat) comparisons. Linear trends between groups were calculated using polynomial coefficients. Statistical significance level was set at $p<0.05$ for all tests. The statistical analyses were computed and analyzed by a certified researcher using the SPSS Statistics 19.0.

TABLE 13: DEFINITION OF THE OPTIMAL CARDIOVASCULAR HEALTH SCORE.

Criteria	Optimal metric definition in this study
Health factors	
Blood Pressure	< sex-specific SBP/height ratio cut-offs for detecting elevated blood pressure in children [265]
Health Behaviors	
Body Mass Index	< sex- and age-specific cut-offs for obesity [108]
Waist circumference	< sex- and age-specific 85th percentile curve for the Portuguese youth [109]
Cardiorespiratory Fitness	≥ recommended level for metabolic health [199]
Muscular Strength	≥ sex- and age-specific 20th percentile [240]

RESULTS

Prevalence of overweight and obesity in this study was 28.7% and 12% of the children had increased WC (TABLE 14). Seventy-two percent of the children were above the 20th percentile of muscular strength. Mean values of absolute and indexed muscular strength were similar between boys (23.36; 7.70) and girls (22.61; 7.80) ($p>0.05$). The recommended CRF level for metabolic health was attained by 61.7% of the children. Mean cIMT was 0.50mm.

Approximately 27% of children had all the optimal health behaviors and health factors after dichotomization according to TABLE 13. The criterion for optimal blood pressure was the least often met criterion (61.5%). The Low Score group had the highest cIMT (0.53mm; $p<0.05$) and cIMT decreased linearly as children scored higher on the optimal cardiovascular health metrics ($p <0.05$) (FIGURE 12).

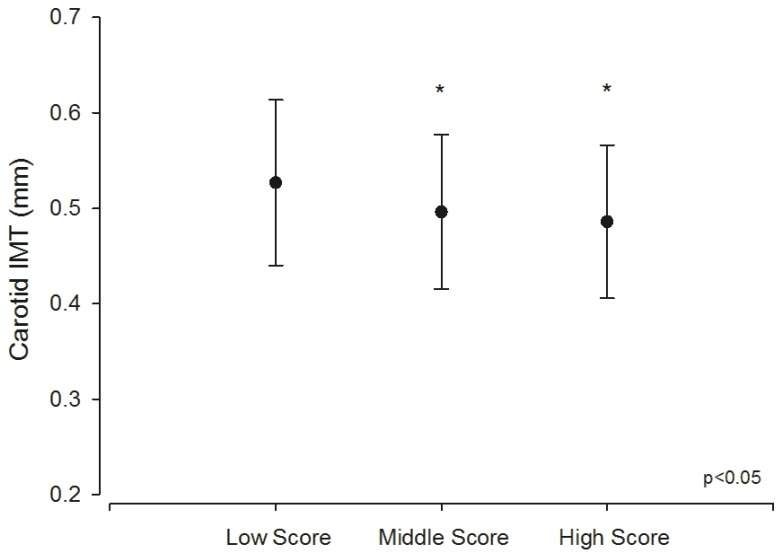

FIGURE 12: CIMT ACCORDING TO THE NUMBER OF OPTIMAL CARDIOVASCULAR HEALTH METRICS (SUM OF HEALTH BEHAVIORS AND HEALTH FACTORS)

* Significant differences from Low Score group ($p<0.05$)

The determinants of cIMT were examined in multivariate regression analyses in the entire cohort (TABLE 15). cIMT was inversely associated with MSI independently of sex, maturity

offset and PP (1st model). This association was also independent of CRF (2nd model) and trunk fat (3rd model).

Physiological differences in CVD risk were examined after categorization of participants into MSI tertiles. The three MSI groups were similar in age, fat and lean soft tissue, maturity offset, chronotropic responses and DBP ($p>0.05$; TABLE 14). The highest relative prevalence of overweight and obesity, high WC and children failing the recommended CRF cut-off values were found in the LMSI group (32.0%, 13.1%, 44.3%, respectively). The lowest prevalence was found in the HMSI group (26.7%; 9.0%, 34.4%, respectively). Children in the LMSI group had the highest cIMT (0.52mm; $p<0.05$; FIGURE 13) and children in the MMSI had the lowest cIMT mean values (0.48mm) although this was not significantly different from that of the HMSI group (0.49mm; $p>0.05$). Increased cIMT in the LMSI does not merely reflect a larger vessel. Indeed, there were no differences in diameter among MSI groups once adjusted for sex and maturity ($p=0.10$). Furthermore, differences in cIMT between LMSI and MMSI and between LMSI and HMSI remained after adjustments for the diameter of the artery ($p=0.001$, $p=0.04$, respectively) and other confounders such as sex, CRF, trunk fat and maturity ($p=0.02$, $p=0.04$, respectively).

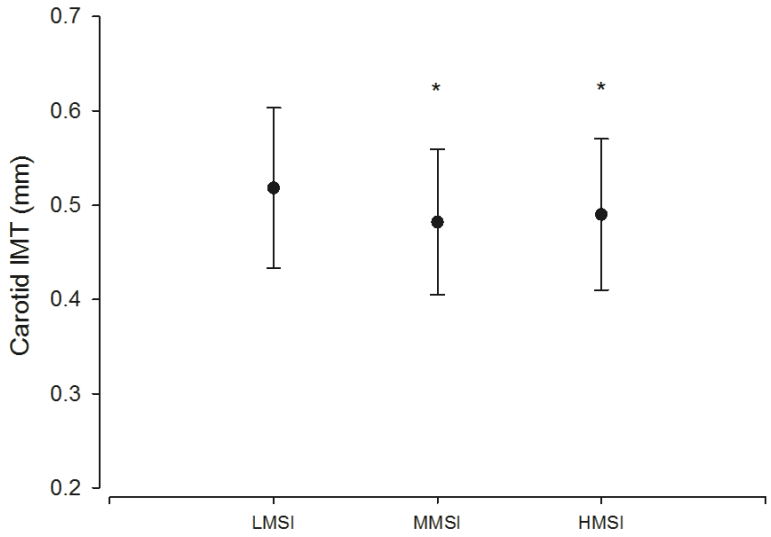

FIGURE 13: CIMT ACCORDING TO MUSCLE STRENGTH INDEX TERTILES.

* Significant differences from LMSI ($p<0.05$)

DISCUSSION

The novel finding from this study was that muscular strength is inversely associated with cIMT independently of sex, maturity, PP, central fat and CRF. Children with low muscular strength have the highest cIMT values, WC and SBP coupled with the low CRF, apparently setting the stage for increased risk of cardiovascular complications in adulthood.

TABLE 14: CHARACTERISTICS OF THE STUDY GROUP AND DIFFERENCES BETWEEN MUSCULAR STRENGTH INDEX GROUPS.

		LMSI	MMSI	HMSI	All
n		122	122	122	366
Age	(years)	11.35 ± 0.48	11.33 ± 0.47	11.37 ± 0.48	11.35 ± 0.48
Girls/Boys	(n)	58/64	63/59	70/52	191/175
BMI	($kg.m^{-2}$)	19.89 ± 3.74	19.45 ± 3.07	19.05 ± 3.39	19.46 ± 3.42
WC	(cm)	68.31 ± 8.17	66.58 ± 6.30	65.15 ± 6.80*	66.68 ± 7.23
Total body fat	(%)	27.05 ± 7.35	27.25 ± 7.19	27.97 ± 7.96	12.68 ± 6.05
Trunk fat	(%)	23.37 ± 8.47	23.04 ± 8.23	23.90 ± 8.86	23.44 ± 8.51
Upper limb body fat	(%)	26.98 ± 10.34	27.49 ± 10.18	28.83 ± 11.09	27.77 ± 10.54
Total body lean soft tissue	(%)	69.91 ± 7.05	69.67 ± 6.90	68.95 ± 7.65	69.51 ± 7.20
Trunk lean soft tissue	(%)	74.80 ± 8.28	75.12 ± 8.04	74.27 ± 8.67	74.73 ± 8.32
Upper limb lean soft tissue	(%)	69.74 ± 10.12	69.16 ± 9.94	67.89 ± 10.90	68.93 ± 10.33
Maturity	(years)	-0.85 ± 1.08	-0.89 ± 1.02	-0.92 ± 1.11	-0.89 ± 1.07
Muscular strength	(kg)	20.84 ± 4.08	23.20 ± 4.36*	24.86 ± 3.91*	22.96 ± 4.43
Rest HR	(bpm)	89.63 ± 14.98	89.56 ± 13.20	89.51 ± 15.25	89.57 ± 14.47
Peak HR	(bpm)	193.55 ± 12.14	195.22 ± 10.67	196.09 ± 9.88	194.95 ± 10.95
CRF	($ml.kg^{-1}.min^{-1}$)	40.86 ± 7.86	42.55 ± 7.88	43.21 ± 8.90*	42.21 ± 8.27
SBP	(mmHg)	112.50 ± 11.36	110.94 ± 9.97	109.54 ± 10.13*	110.99 ± 10.55
DBP	(mmHg)	62.05 ± 8.36	61.40 ± 8.52	61.61 ± 7.79	61.69 ± 8.21
Carotid diameter	(mm)	6.33 ± 0.49	6.29 ± 0.50	6.16 ± 0.46*	6.26 ± 0.49

* Significant differences from LMSI ($p<0.05$)

The results of the present study extend those of Artero et al. [266] and Steene-Johannessen et al. [267], the latter demonstrating that muscular strength was independently and inversely associated with clustered metabolic risk in a cohort of 9- and 15-yr-old Norwegian children (N = 2818). Others [258, 259] have also shown that greater muscular strength (N/kg) in youth is associated with lowers levels of CVD risk factors, coupled with better insulin sensitivity and beta-cell function in young adulthood. Because initiation of muscle-strengthening activities can lead to gains in muscular strength [268], these observations indicate that it also has the potential to offer observable vascular value beyond that of CRF [233] and other traditional CVD risk factors [97].

This suggests that low muscular strength is causally related to development of unfavorable levels of CVD risk factors [258], in particular increased cIMT. In fact, we ran a supplementary multiple linear regression analysis (data not shown) to estimate the R^2 change from a model with cIMT as the dependent variable and BMI and cardiorespiratory fitness as independent variables, to a second model where MSI entered as an additional independent variable, both models fully adjusted for sex, maturity offset and PP. A significant R^2 change ($p<0.05$) was observed when MSI was added. Thus, handgrip strength may be a useful tool in clinical settings and for preventive services in schools, with good reliability without additional costly equipment [257].

TABLE 15: MULTIPLE REGRESSION ANALYSIS WITH CIMT AS DEPENDENT VARIABLE AND PHYSICAL FITNESS AND ABDOMINAL FAT AS DETERMINANTS.

cIMT	Variables	R^2	Beta	95% CI	
Model 1	MSI	0.05	-0.11	-0.22	-0.01
Model 2	MSI	0.05	-0.11	-0.21	-0.01
	CRF		-0.05	-0.17	0.07
Model 3	MSI	0.06	0.12	-0.22	-0.01
	CRF		0.01	-0.15	0.17
	Trunk fat		0.09	-0.06	0.23

All models were adjusted for sex, maturity offset and PP.

Only 27% of the children in this study had optimal cardiovascular health scores. Others have found similar low prevalence of ideal cardiovascular health in youth [269]. Previous studies in adults have also reported a very low or non-existing prevalence of ideal cardiovascular health [270]. Based on our findings, vascular health benefits are gained by the avoiding a low health score. Consequently, we share Pahkala's et al. [271] view that not all the ideal metrics are needed to gain cardiovascular health benefits in childhood. The cIMT increased by 0.04 mm in children with two or less optimal metrics is disconcerting considering how difficult it is to reduce cIMT through interventions [63].

The differences in the LMSI group in cIMT, CRF, WC and blood pressure translate to physiological differences increasing CVD risk. The apparent protective effect in the higher MSI groups may be a function of insulin. Several cross-sectional studies among youth uncovered an inverse association between muscular strength and insulin resistance independent of CRF [259, 267]. In addition, small scale trials have been conducted among youth comparing the effect of resistance training on insulin resistance or glycemic control with a control group concluding that not only does this type of training increase strength and lean mass but it also had benefits on insulin sensitivity and slightly reduced glucose production [272, 273]. Hyperinsulinemia driven by insulin resistance promotes cardiovascular pathology, stimulating mitogen-activated protein kinase, mitogenesis, and PAI-1 within vascular smooth muscle cells [274], endothelin-1 production with subsequent vascular smooth muscle growth [275], and ras-p21 in vascular smooth muscle, which promotes a cascade of other growth factors such as platelet-derived growth factor. Thus, interventions designed to prevent hyperinsulinaemia and related metabolic disorders should focus not only on reducing fatness but also improving muscular strength [266]. Although this study cannot evaluate causal pathways, children with increased cIMT should be encouraged to engage in exercise programs and other forms of physical activity that complement aerobic endurance training with muscle performance.

STRENGTHS AND LIMITATIONS

This study has several strengths and limitations:

An important strength of the current study was that we were able to examine the independent associations of muscular strength and cIMT, as well as control for important

confounding factors such as CRF and body composition which were measured objectively. Automated edge-detection on the basis of RF signal processing of B + M mode US imaging is probably the most accurate method to measure cIMT [107]. The large number of participants in this study suggests that results are representative of the population tested.

The gold-standard measure of CRF in humans involves direct assessment of maximal oxygen consumption in response to an exercise test. In this study CRF was indirectly determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer. Although our concept of cardiovascular health score did not meet all the criteria used in defining ideal cardiovascular health by the American Heart Association [264] we took into consideration similar concepts in health promotion and disease prevention: (1) The power of primordial prevention and (2) the evidence that CVD and risk factors for it often develop early in life. These concepts informed our definition of optimal cardiovascular health, as well as the metrics that would be needed to monitor it, but this was only a scientific exercise. We calculated MSI dividing peak absolute force by an objective measure of body composition. However, we should consider that imaging techniques like DXA are not available for large-scale use. Validated sex-specific anthropometric formulas to calculate lean body mass may aid in accounting for lean body mass in future studies. This was a cross-sectional study, and we did not measure insulin resistance or beta-cell function so the biological plausibility presented in this study can only be inferred. There are no data per se indicating an increased risk of mortality in children with higher than normal cIMT, probably as a function of extremely low mortality rates due to atherosclerosis in children. Also, there is currently no longitudinal evidence tracking cIMT from childhood to adulthood, evaluating if high cIMT in childhood confers increased risk in adulthood. Still, there is evidence that atherosclerosis may start at an early age [276]. To evaluate early, subclinical disease, assessment of cIMT has been used extensively in children and young adults with known risk factors for cardiovascular disease. Increased cIMT relative to normal children has been demonstrated in pediatric patients with CVD risk factors [79]. In randomized clinical trials assessing statin therapy in children [39, 91], diet, and exercise [35], cIMT decreased in accordance with changes in other cardiovascular risk factors, whereas cIMT in the placebo groups increased, suggesting that cIMT is indeed a surrogate marker of atherosclerotic burden in children.

The developmental changes of cIMT during the developmental years have not been clearly elucidated [277, 278]. If cIMT changes throughout childhood, these changes are very small [35] and we understand that their clinical or functional relevance are truly questionable. We are also aware that these changes in the IMT are accompanied by increases in arterial size, including luminal diameter [52, 277]. Therefore, it is uncertain whether some of the changes in cIMT that occur with age represent normal vascular adaptation or a pathological change [79].

CONCLUSION

There is an inverse and independent association between muscular strength and cIMT. Low muscular strength is also associated with higher levels of CVD risk factors in children. From a public health perspective, a handgrip strength test may be a useful tool for designing preventive services in clinical and school settings.

INSIGHTFUL DISCUSSIONS WITH PEERS

The major criticism of the study that should be addressed by the authors is the assumption that the measured cIMT in children reflects subclinical atherosclerosis with prognostic value in prediction of cardiovascular events. In the systematic review and meta-analysis cited [133], the study populations were much older with the lowest age group down to 19 year-old subjects. I would therefore suggest that the authors provide data or evidence that cIMT in children is a valid marker of subclinical atherosclerosis which can predict cardiovascular events.

We agree with the Reviewer that there is a lack of evidence that carotid intima-media thickness (cIMT) is associated with atherosclerotic mortality in children. However, based on the evidence presented below, we believe that cIMT is indeed a valid marker of atherosclerotic burden in children.

cIMT is a non-invasive ultrasound biomarker of early atherosclerosis in adults. A positive association exist between cIMT and the risk of subsequent cardiovascular events in general adult populations, independent of all major risk factors [133]. This relation has promoted the use of cIMT in pathophysiological studies and clinical trials, in which the

perception of cIMT has shifted from a secondary endpoint to a surrogate of risk of cardiovascular event in adults [31]. Many studies already include the assumption that relations with cIMT, as seen in the general population or risk cohorts, reflect associations with the risk of cardiovascular events in adults [279-281].

Although clinical cardiovascular heart disease occurs in later life, there is evidence that atherosclerosis may start at an early age [276]. To evaluate early, subclinical disease, assessment of cIMT has been used extensively in children and young adults with known risk factors for CVD. Increased cIMT relative to normal children has been demonstrated in pediatric patients with familial hypercholesterolemia [38, 39, 44, 91, 282], hypertension [42, 43], obesity [34, 93], type 1 diabetes mellitus [44, 45], and the metabolic syndrome [95]. In randomized clinical trials assessing statin therapy in children [39, 91], diet, and exercise [79], cIMT was decreased in accordance with changes in other cardiovascular risk factors, whereas the cIMT increased in the placebo groups, suggesting that cIMT is indeed a surrogate marker of atherosclerotic burden in children. However, it should be noted that there are no data per se indicating an increased risk of mortality in children with higher than normal cIMT, probably as a function of extremely low mortality rates due to atherosclerosis in children. Also, there is currently no longitudinal evidence tracking cIMT from childhood to adulthood, evaluating if high cIMT in childhood confers increased risk in adulthood.

The developmental changes of cIMT during the developmental years have not been clearly elucidated [277, 278]. These different findings are probably related to the very small changes observed in cIMT between 7 and 18 years of age [35]. If cIMT changes throughout childhood, these changes are very small (16/1000 mm) and we understand that their clinical or functional relevance are truly questionable. We are also aware that these changes in the cIMT are accompanied by increases in arterial size, including luminal diameter [52, 277], suggesting that the increase in cIMT may be a function of increased overall arterial size [54, 283]. Therefore, it is uncertain whether some of the changes in cIMT that occur with age represent normal vascular adaptation or a pathological change [79]. Nevertheless, Bots et al [284] compared end-diastolic lumen to cIMT to study the relationship between shear stress and transmural pressure and found that beyond a certain level, cIMT did indicate true atherosclerotic change.

Other methods, such as coronary arteriography and intravascular coronary ultrasound studies, confirm disease at a young age, but are not applicable for large population studies nor can they be recommended for asymptomatic individuals. On the other hand, carotid ultrasound measurements are relatively easy to obtain and can be a marker for the intensity of the long-term burden of cardiovascular risk factors beginning at a young age. Understanding risk factors at a young age and observing their effects on the cardiovascular system can guide early prevention modalities [285].

Since the authors suggest the use of handgrip strength test it would be useful if the authors could provide data that would suggest that measuring muscular strength index in children has additional value compared to the traditional risk factors in primary prevention of CVD.

We agree with the suggestion of the Reviewer that the additional value of measuring muscular strength should be clarified and we believe the arguments presented below go in that sense:

Knowledge about different risk factors at early stages of life is needed for disease prevention and early treatment. Body mass index has served the obesity research and clinical community very well over the last few decades. The link between excess fatness and health indicators as well as mortality rates was established through hundreds of epidemiological and randomized controlled studies that used BMI and body weight fluctuations. The evidence was deemed so strong that it convinced World Health Organization to emphasize the assessment of BMI at younger ages, providing different standards according to age and sex [286]. In addition to traditional risk factors, such as obesity and a low CRF level during middle or older ages [287, 288], low muscular strength has been proposed as a predictor of premature all-cause mortality as well as mortality due to CVD in adults [245, 256, 289] and in adolescences [257]. The latter study suggests that muscular strength in adolescence is as important as BMI in terms of all-cause mortality, whereas BMI has a greater role than muscular strength in terms of mortality due to CVD.

Based on the evidence presented above and on the previously reported association between BMI and cIMT [97] and CRF and cIMT [233] in children, the authors ran a supplementary multiple linear regression analysis to estimate the R^2 change from a model

with cIMT as dependent variable and BMI and CRF as independent variables, to a second model where muscular strength index entered as an additional independent variable, both models fully adjusted for sex, maturity offset and PP. A significant R^2 change ($p<0.05$) was observed when muscle strength index was added, thus increasing the explanatory power of BMI over cIMT. Accordingly it seems that handgrip strength can be a useful additional tool for clinical settings and for preventive services in schools, with good reliability in almost any place without additional costly equipment [257]. Although this study cannot disentangle causal pathways, children at increased risk of early subclinical atherosclerosis should be encouraged to engage in exercise programs and other forms of physical activity that complement aerobic endurance training with muscle performance.

CHAPTER 2

ARTERIAL STIFFNESS

1. BACKGROUND

Arterial compliance, distensibility, and stiffness are well-studied markers of structural change of the arterial properties. Although both compliance and distensibility can be used as measures of stiffness, they individually represent different facets of arterial structure and function [79]. Distensibility is a measure of the elastic properties of an artery given by the relative change in diameter with pressure, whereas compliance is a measure of the local vessel capacity to respond to changes in blood volume given by the absolute change in diameter with pressure [290]. Arterial stiffness is the reciprocal of distensibility.

Arterial stiffness is a dynamic property that is dependent on vascular structure, function and pressure. These factors function independently and in concert to effect changes in arterial stiffness. Laurent et al. [291] has shown that distensibility and compliance of the radial artery of hypertensive patients were not significantly different from those of normotensive controls when the two populations were studied at their MAP. This suggests that arterial pressure is a major determinant of arterial stiffness, and elevated arterial pressure increases arterial stiffness. A progressive increase in HR, stroke volume, elevated vascular resistance, and early wave reflections are also accompanied by marked reductions in carotid artery compliance and distensibility [292].

The central arteries store more elastic energy during systole as a result of the high elastin-to-collagen ratio and decreased influence of smooth muscle tone. Wall stress and stiffness increases toward the peripheral vessels as the ratio of elastin to collagen in the wall declines and the effect of smooth muscle contraction and its associated series collagen becomes increasingly important [293].

RELATIONSHIP TO CARDIOVASCULAR RISK IN ADULTS

Increasing stiffness is an antecedent factor in hypertension [294] and PP, suggesting that the former is a risk factor for the latter [295]. Thus, it is strongly associated with the presence and extent of atherosclerosis and constitutes a forceful marker and predictor of cardiovascular risk in adults [296, 297].

Decreases in arterial distensibility have been shown in adult populations with hypertension [298], diabetes mellitus [299] and are more prevalent with clustering of cardiovascular risk factors [300].

Aortic PWV is strongly associated with the presence and extent of atherosclerosis [296] and is increased in the presence of various cardiovascular risk factors, including diabetes [301], hypertension [302], end-stage renal disease [303], hyperlipidemia [304], increasing age [305], and sedentary lifestyle [306].

RELATIONSHIP TO CARDIOVASCULAR RISK IN CHILDREN AND ADOLESCENTS

Arterial distensibility measured with ultrasound is impaired in the settings of positive family history of myocardial infarction [307], elevated total and low-density lipoprotein cholesterol [308], obesity [309, 310], elevated leptin levels [311], increased blood pressure [312, 313], and type 1 diabetes mellitus [314]. Compliance and elasticity of the carotid artery was also shown to be impaired in obese children [36, 315], although it is not consensual [102]. Hypertension also correlates closely with arterial compliance. Decreases in elastic properties of the carotid artery were found to be reduced in children in the upper race-, sex-, and age-specific tertile for both serum total cholesterol and SBP [307]. However, due to the lack of adjustments it remains unclear whether it was blood pressure, lipid profile or obesity that contributed the most to the differences in arterial compliance observed. Litwin et al. [313] showed that the lumen diameters of the carotid artery were greater in hypertensive children and that the mechanical properties of the arterial wall, were also deranged. Distensibility was significantly decreased in the hypertensive group but compliance in contrast was increased. The higher diameter of the artery in hypertensive patients may explain the greater compliance of the artery suggesting that hypertension must last sufficiently long to cause remodeling of the arterial wall, which will further reduce compliance of the artery.

The majority of the available PWV research in pediatric subjects includes those with type 1 diabetes mellitus [316], neurofibromatosis [317], Kawasaki disease [318], polyarteritis nodosa [319], and coarctation of the aorta after surgical repair [320]. In community-based children, BMI, WC, and TBF were independently and positively associated with PWV after

adjusting for age, sex, SBP, MAP, HR, and CRF. However, the influence of CRF on PWV was attenuated after adjusting for fatness [321]. In contrast, obese adolescent girls were shown to have lower PWV and higher arterial diameters compared to lean adolescent girls, despite increased radial intimal thickness [322]. The authors suggest that the decreased PWV in the obese versus lean subjects could reflect general vasodilatation stimulated by hyperinsulinemia associated with obesity and relative insulin resistance. Additionally, the higher arterial compliance in childhood obesity might indicate developmental change associated with maturation [78].

Correlations with MAP and PP were demonstrated in normotensive, non-obese adolescents evaluated with brachial-ankle PWV with the intent of establishing mean PWV values for further comparison studies [323]. Sex and racial differences in brachial-ankle PWV were present, with males having values greater than females and black subjects having values greater than white subjects [323].

DEVELOPMENTAL ASPECTS OF ARTERIAL STIFFNESS

Carotid stiffness increase throughout childhood and adolescence [52, 53, 324, 325], with the fastest decline observed during the first few years of life. Although the trend is clear, the amount of change appears to be variable between studies. Carotid compliance decreases by 10–28% between 5 and 20 years of age, coupled with increases in elastic modulus and stiffness index β of 9–30% [35]. The age-associated increase in arterial wall stiffness in children is also supported by several studies showing increases in both aortic and peripheral PWV from childhood through puberty [305, 319, 326, 327].

The increase in arterial capacitance suggests that arterial buffering capacity increases independently from changes in arterial wall elasticity in children, since both elastic modulus and stiffness index β continually increase throughout childhood [325]. Thus the increase in arterial size appears to offset the increase in wall stiffness, preventing an increase in afterload that could adversely affect ventricular performance [328-330]. This is different from the effect of aging in adults, where arterial capacitance decreases concomitant with an increase in arterial wall stiffness [305, 326].

EFFECTS OF INTERVENTION

In adults, 30-40 minutes of moderate to vigorous aerobic exercise training most days of the week improves blood pressure, appears to significantly reduce augmentation index and improve carotid artery compliance. Resistance training and combined exercise training also appears to significantly improve blood pressure and appears to have some benefits for vascular function. However, further studies are necessary to strengthen those findings with most studies finding aerobic exercise to have more consistent effects [331].

The limited work on the relationship between physical activity and arterial compliance in healthy children has provided contradictory findings. The amount of time children spend in play or sport participation as assessed by questionnaire was inversely related and independently contributes to the arterial stiffness of both the aorto-radial and the aorto-femoral segments [227]. In contrast, Reed et al. [229] found no significant relationship between the total estimated amount of physical activity and arterial compliance in children. These disparate results may reflect a weak relationship between physical activity and arterial compliance in healthy children or known difficulties in accurately estimating physical activity levels [35].

Cross-sectional research suggest that aerobic fitness may contribute to improved arterial compliance in healthy children [229] but studies are scarce. The number of laps completed on a 20-m shuttle-run was positively associated with small and large arterial compliance in 9-11-year-old children, supporting the concept that physical fitness may exert a protective effect on the cardiovascular system [229]. In contrast, highly trained pre-pubertal swimmers demonstrated higher upper-limb PWV and lower pressure-corrected index of distension compared with age- and sex-matched non-swimmers [332]. Furthermore, the child swimmers showed PWV values similar to those of adults, suggesting an early detrimental effect of high-volume swim training on upper-limb arterial stiffness in children. The authors suggest that the higher upper-limb PWV in highly trained pre-pubertal swimmers may be related to enhanced smooth muscle content of the arterial wall, possibly due to intermittent elevations in arterial blood pressure during repetitive swimming exercise sessions. Nonetheless, these findings underscore the importance of considering the type of exercise stimulus in evaluating the effect of exercise training on arterial compliance in youth. It is also evident that the mode, volume, and duration of exercise

training that may improve arterial stiffness and endothelial function in healthy children remain largely unknown [35].

Although weight loss has been shown to improve arterial compliance [333], carotid arterial distensibility [334], and endothelial function [335], further studies are required to evaluate whether public health efforts to promote physical activity and weight loss in children will reduce arterial stiffness, attenuate the progression of subclinical cardiovascular disease, and prevent the development of subsequent cardiovascular events in the community [321].

POSSIBLE UNDERLYING MECHANISMS

One of the most important molecular consequences of regular physical exercise is the absolute increase of vascular nitric oxide concentration. Nitric oxide is responsible for vasodilation, which results in the lowering of peripheral resistance and increase of perfusion. Endothelial nitric oxide synthase, the main source of nitric oxide, is up-regulated by an increase in flow-mediated shear stress associated with physical exercise due to a complex pattern of intracellular regulation like acetylation [336], phosphorylation [337], and translocation to the caveolae [338]. It now clearly documented that exercise or increased shear stress up-regulates endothelial nitric oxide synthase activity in humans [339]. Nevertheless, it remains unclear how an elevated shear stress is translated into increased endothelial nitric oxide synthase activity [340].

Structural changes, in particular collagen and elastin, are also thought to play a central role in the development of arterial stiffness. However, studies investigating the impact of different modes of exercise training on these structural components in animal models are conflicting [341, 342].

Advanced glycation end-products (AGEs) accumulate with age, leading to the cross-linking of collagen and subsequently to arterial stiffness [340]. Additionally, AGEs also stimulate pro-inflammatory mechanisms, increase production of superoxide anions, affect endothelial-mediated smooth-muscle function, and increase oxidative stress [343-345]. With respect to exercise training, a significant inverse relationship between AGEs content in the skin and muscle strength was observed in humans [346], and a long-term exercise training decreased plasma levels of carboxymethyl-lysine as well as inhibited age-related cross-linking of collagen in the heart muscle in animal models [347].

ARTERIAL STIFFNESS APPLIED TO THE ACUTE EXERCISE MODEL

The phenomenon of exercise-induced changes of arterial compliance is a largely understudied field of clinical significance, especially in adults with CVD in which arterial compliance plays an important role, but also in children and adolescents, once considered to be at low risk, but with the growing health concerns related to sedentary lifestyle, poor diet and obesity, vascular screening may be needed earlier so that interventions to improve cardiovascular health can be initiated. Although arterial compliance has been used in intervention trials evaluating the salutary effect of drugs, supplementation [348] and lifestyle modifications [349, 350] in children, until recently researchers have not used arterial stiffness to examine the role of a single bout of exercise on the arterial tree. It is possible that the effects of a bout of exercise can predict the effects of chronic exercise (accumulation of single bouts of exercise over time), as is the case in several variables, blood pressure reduction being a prime example [351].

The acute exercise model can be useful in investigating mechanisms of the exercise (acute or chronic) response [352]. Although the number of investigations reporting arterial stiffness following exercise is limited even in adults, the purpose for the study of post-exercise arterial stiffness and the diversity of the populations studied are not. Oxidative stress [353], arterial response to acute exercise [354], postprandial arterial stiffness [355], systemic and regional hemodynamics [356], smoke exposure [357] and muscle ischemia [358] have been the focus of studies utilizing arterial stiffness in the acute model.

Utilizing the acute exercise model can be advantageous as it allows for an efficient manipulation of exercise variables (i.e. mode, intensity, duration, etc.) and permits greater experimental control of confounding variables. Incremental exercise to exhaustion [354, 359-361], moderate intensity continuous exercise [328, 362], brief low intensity exercise [363, 364] and supramaximal exercise [365, 366] are some of the varying protocols used to examine changes in central and peripheral arterial distensibility measurements conducted at discrete time points ranging from 2 min [365] to 240 min [362] following exercise. In addition, it is also possible to unmask vascular abnormalities that are not present at rest using acute physical stress [357].

2. Purpose

Whereas several studies have been performed to compare cardiovascular adaptations between children and adults during maximal exercise [367-371], no study has yet compared large artery distensibility adaptations to exercise in children and adults, although it is a physiological important indicator of cardiovascular efficiency [359]. To date, cardiovascular adaptations in children have been assessed with cardiac output, stroke volume, left ventricular dimensions and HR kinetics in relation with the work rate or oxygen uptake. These preliminary studies suggest that peak cardiac responses to exercise are not age-related when expressed relative to work intensity and body size [369-372]. It is clear that there are inherent age differences related to arterial compliance at all age levels, but the underlying mechanisms that explain these differences remain speculative. It is conceivable that examination of the response of the arteries to physical stress, such as acute intense exercise, could offer additional critical information about vascular differences. Therefore, the objective of the current chapter of the dissertation was to investigate differences in arterial stiffness and hemodynamic parameters in children and adults at rest and after acute physical stress.

The research carried out on arterial compliance as part of the present doctoral research program resulted in the following publication, and communications (oral/poster) as first author:

PEER-REVIEWED ARTICLES THAT ARE RELATED TO ARTERIAL STIFFNESS

Melo, X., Fernhall, B., Santos, DA., Pinto, R., Pimenta, NM., Sardinha, LB., Santa-Clara, MH. *The Acute Effect Of Maximal Exercise On Central And Peripheral Arterial Stiffness Indices And Hemodynamics In Children And Adults.* Submitted to Applied Physiology, Nutrition, and Metabolism, 2015 (1st revision). JCR Impact Factor: 2.225 (2013). JCR Rank: Q1 (Sports Science).

ABSTRACTS THAT ARE RELATED TO ARTERIAL STIFFNESS

Melo, X., Fernhall, B., Santos, DA., Pinto, R., Pimenta, NM., Sardinha, LB., Santa-Clara, MH. *Age-Based Comparison Of The Acute Effect Of Maximal Exercise On Arterial Stiffness In Children And Adults.* In: ARTERY, 2014. Maastricht. ARTERY Research. Philadelphia PA: Elsevier For Official Journal Of The Association For Research Into Arterial Structure And Physiology, 2014, v. 8.

Melo, X., Fernhall, B., Santos, DA., Pinto, R., Pimenta, NM., Sardinha, LB., Santa-Clara, MH. 2015. *Carotid Artery Elastic Function And Hemodynamic Changes Following Maximal Exercise In Children And Adults.* In: 62th Annual Meeting, 6th World Congress On Exercise Is Medicine, And World Congress On The Basic Science Of Exercise Fatigue Of The American College Of Sports Medicine, In Medicine And Science In Sports And Exercise, 2015 San Diego.

3. METHODS OF ASSESSMENT OF ARTERIAL STIFFNESS

Three groups of noninvasive methods are typically used in the assessment of arterial stiffness: 1) analysis of the arterial pressure waveforms; 2) calculation of the change in diameter (or area) of an artery with respect to the distending pressure; and 3) measurement of pulse-wave velocity (PWV), which appears to be emerging as the gold standard in studies of adults [79]. Here we discuss some. More detailed methodological procedures may be found in the article.

ARTERIAL DIAMETER CHANGE

Measurement of the change in arterial diameter as it relates to distending pressure provides a reciprocal of arterial stiffness, defined as arterial distensibility. As stated, arterial distensibility is a measure of the elastic properties of an artery [79]. Distensibility describes the amount of diameter expansion expressed as a percentage of the initial diameter of the artery in relation to the force that causes the expansion (transmural pressure) [290]. This change in diameter can be measured by radiofrequency tracking of a regional arterial segment such as the carotid, brachial, radial, or femoral artery [79].

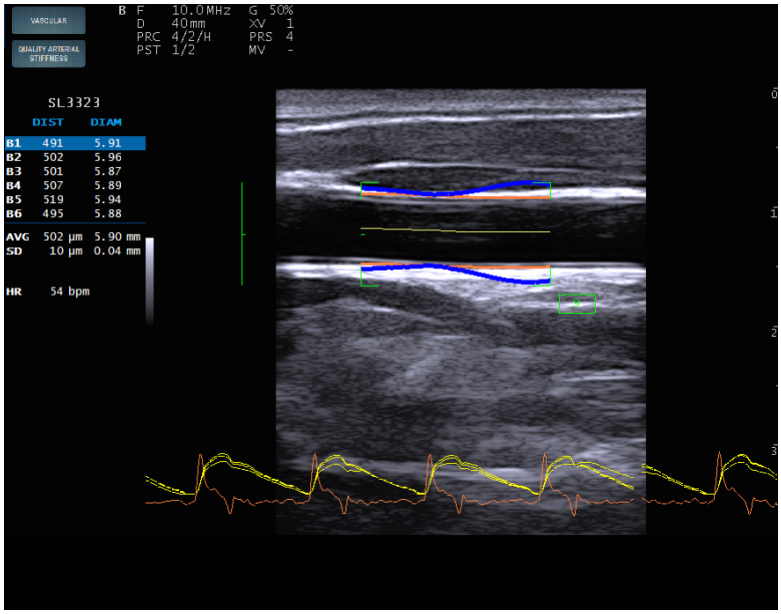

FIGURE 14: ULTRASOUND EVALUATION OF DISTENSIBILITY WITH RADIOFREQUENCY ECHOTRACKING.

The real vessel walls movement are "amplified" by a predefined factor. This means that what the blue lines are displaying is not the real displacement according to the B-Mode image size, but it is a relative indication for the clinician to estimate at first sight the distension.

We used an ultrasound scanner equipped with a 13 MHz linear probe (MyLab One, Esaote, Italy) with Quality Arterial Stiffness technology, based on radiofrequency signal in a common carotid artery segment ~1 cm before the bifurcation (FIGURE 14). This software used a complex algorithm that could process all data coming from the arterial wall as radiofrequency signals, automatically measuring the modification of the arterial diameter between the systolic and diastolic phases.

Carotid diameter waveforms were then converted to carotid pressure waveforms using an empirically derived exponential relationship between pressure and arterial cross-section. The derived carotid pressure waveform is calibrated to brachial end DBP and MAP by iteratively changing the wall rigidity coefficient. This allows the calculation of carotid stiffness indices: pulse wave velocity (m/s), distensibility coefficient (1/KPa), compliance coefficient (mm^2/kPa), stiffness index α and β.

PULSE WAVE VELOCITY

The use of PWV as an index of cardiovascular health has increased tremendously, and it is recognized as likely the best clinical measure of stiffness over an arterial segment [373]. PWV, as a measure of arterial stiffness, is based on the principle that the pressure pulse, generated by ventricular ejection, is propagated along the arterial tree at a speed determined by the geometric and elastic properties of the arterial wall [374].

Several methods of measuring PWV have been described, including the use of pressure transducers [375], Doppler ultrasound [376], applanation tonometry [377], magnetic resonance imaging [378], and oscillometric pressure cuffs [379]. The arterial pulse wave is measured at both a proximal and a distal artery, such as the common carotid and femoral arteries, respectively. These relatively superficial arteries readily allow for noninvasive measurements of arterial waveforms. The pulse waveform is recorded at each site, and the time delay between the arrival of a predefined point on the waveform (typically the "foot") at the 2 sites is obtained by gating to the peak of the R wave on the ECG. Measurement of distances on the body surface, a practice somewhat dependent on body habitus, allows an estimate of the distance traveled. PWV is then calculated as distance/time (m/s). PWV as assessed by ultrasound is defined by the Moens-Korteweg equation as $PWV=\sqrt{(E\mathit{h}/2\rho\mathit{R})}$, where E is Young's modulus of the arterial wall, *h* is wall thickness, ρ is blood density, and *R* is arterial radius at the end of diastole.

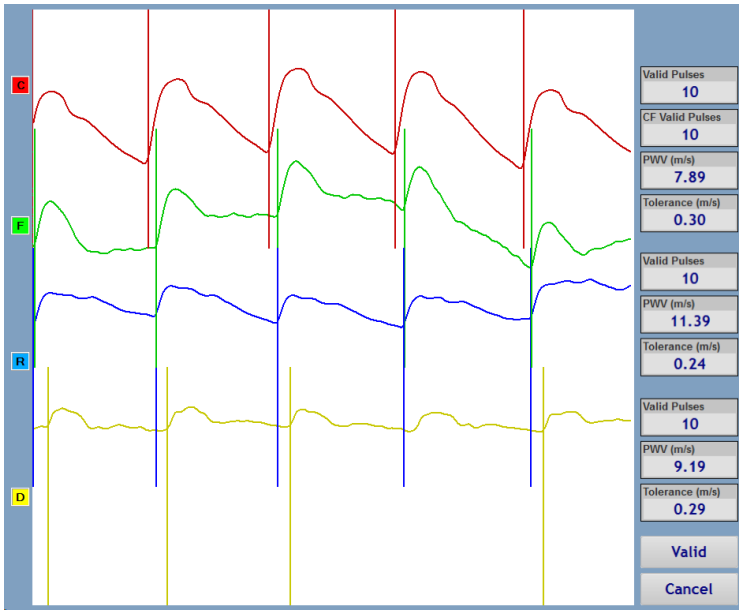

FIGURE 15: THE IMAGE DISPLAYS THE CAROTID, FEMORAL, RADIAL AND DISTAL POSTERIOR TIBIAL ARTERIES.

Ten quality carotid pulse waveforms, and simultaneous carotid and femoral, radial and distal posterior tibial pressure curves were recorded.

In this dissertation PWV was measured by ultrasound derived carotid pressure waveform as referred above, but also by applanation tonometry using the Complior Analyse software (ALAM Medical, Paris, France). In the latter, the carotid and radial sensors, with the help of a specific holder, were positioned and a manually held sensor was placed on the femoral and distal posterior tibial arteries (FIGURE 15). The time delay (aortic transit time) between the two pulse waveforms was calculated automatically according to the intersecting tangent algorithm [380]. The distance traveled by the pulse waveforms was measured between the two recording sites directly on the body surface and was corrected automatically [381]. PWV was then calculated using measurements of transit time and distance traveled by the pulse wave between the two recording sites.

4. THE ACUTE EFFECT OF MAXIMAL EXERCISE ON CENTRAL AND PERIPHERAL ARTERIAL STIFFNESS INDICES AND HEMODYNAMICS IN CHILDREN AND ADULTS

Melo, X., Fernhall, B., Santos, DA., Pinto, R., Pimenta, NM., Sardinha, LB., Santa-Clara, MH. Submitted to Applied Physiology, Nutrition, and Metabolism, 2015. JCR Impact Factor: 2.225 (2013). JCR Rank: Q1 (Sports Science).

ABSTRACT

BACKGROUND: Acute aerobic exercise increases arterial compliance in adults. However, the systemic effect of increases in compliance along the arterial tree after a single bout of aerobic exercise may differ with age.

PURPOSE: The purpose of this study was to compare the effects of a bout of maximal running exercise on arterial elasticity/stiffness in children and adults, controlling for distending pressure changes and body size.

METHODS: Right carotid blood pressure and artery elasticity/stiffness measured by pulse wave velocity (PWV), compliance (CC) and distensibility (DC) coefficients, stiffness index α and β (echotracking), and contralateral carotid blood pressure, upper limb (carotid-radial; CR), lower limb (carotid-distal posterial-tibial; CD) and central/aortic (carotid-femoral; CF) PWV (applanation tonometry) were taken at rest and 10 min following a single bout of a maximal treadmill run in 34 children (7.38±0.38 years) and 45 young adults (25.22±0.91 years) having a similar aerobic potential. 2x2 repeated measures analysis of variance (ANOVA) and covariance (ANCOVA) were used to detect differences with exercise between groups.

RESULTS: Carotid pulse pressure (PP; η^2=0.394) increased more in adults after exercise ($p<0.05$). CC (η^2=0.385) decreased following exercise in particular in adults and in those with high changes in distending pressure similarly to stiffness index α and β. LC PWV increased more in adults and was related to local changes in distending pressure but not changes in mean arterial pressure. Stiffness in the lower limb decreased following exercise (η^2=0.115) but apparently only in those with small mean arterial pressure changes

(η^2=0.111). No significant exercise or group interaction effects were found when variables were adjusted to height.

CONCLUSIONS: An acute bout of maximal exercise can alter arterial stiffness and hemodynamics in vasculatures perfusing the brain and within the active muscle beds. Arterial stiffness and hemodynamic response to metabolic demands during exercise in children simply reflects their smaller body size and may not indicate a particular physiologic difference compared to adults.

KEYWORDS

Arterial Compliance; Pulse Wave Velocity; Rest and Recovery; Height; Distending Pressure

INTRODUCTION

Large artery distensibility is physiologically important for cardiovascular efficiency. Distensible large arteries reduce impedance to systolic ejection and cardiac work, slows pulse wave velocity (PWV) so that the return of reflected pressure waves is delayed until after aortic valve closure, and favors coronary perfusion during diastole [359]. Conversely, stiffening of the central arteries results in an elevation in systolic blood pressure (SBP) and a lowering of diastolic blood pressure (DBP) which, in turn, increases left ventricular afterload and alters coronary artery perfusion [382]. These changes may result in left ventricular hypertrophy [383] and increased fatigue of arterial wall tissues [384], all of which substantially increase the risk of cardiovascular events [385].

The acute effects of exercise on both peripheral and central arterial distensibility in healthy adults have been examined [328, 354, 359-361, 363, 364]. Both acute aerobic and resistance exercise reduce arterial stiffness in the exercised limb, despite having no effect on arterial properties of the non-exercised limb [363, 386]. As for central stiffness, it has been originally suggested that positive alterations were observed with aerobic exercise training whereas potentially negative alterations occur following resistance exercise training [360]. However, the varying exercise protocols used in these studies including incremental exercise to exhaustion [354, 359-361], moderate intensity continuous exercise [328, 362], brief low intensity exercise [363, 364] and supramaximal exercise [365, 366]

showed that the effects of exercise in central stiffness are dependent on the intensity of exercise as well. High-intensity sprint exercise for instance, increases central artery stiffness [365], similar to the response following acute resistance exercise [360, 387].

Acute increases in artery distensibility may be mechanistically linked to the increased arterial distensibility observed with chronic exercise training [359]. To date no study has determined whether the physiological and morphological specificities of pre-pubertal children have an impact on their vascular responses to acute maximal exercise. Furthermore, it is possible that if changes in arterial stiffness indices following high-intensity exercise in adults are related to changes in blood pressure [366], the arterial response may be different in children because they have lower SBP during maximal exercise compared to adults [370]. Whether the change in arterial stiffness indices following high-intensity exercise in children are a function of central or peripheral distending pressure is also unknown. Considering that central blood pressure may be different than standard brachial blood pressure [387, 388], it is essential that an appropriate central blood pressure is obtained.

The purpose of this study was to compare the effects of a bout of maximal running exercise on local, central and peripheral arterial stiffness indices and hemodynamic response in children and young adults, controlling for exercise induced changes (Δ) in distending pressure and body size differences.

METHODS

Thirty-four children 5 to 10 years of age (7.38±0.38 years; 50% females) and 45 young adults 18 to 36 years of age (25.22±0.91 years; 47% females) participated in this study. All participants were free from cardiovascular, respiratory and metabolic diseases and none was considered to be a trained athlete. The Ethics Committee of the Faculty of Human Kinetics - University of Lisbon approved the study. Children provided assent for their participation and informed consent was obtained from their legal guardians. Informed consent was obtained from all adult participants.

The study population was sequentially studied between January 2013 and June 2014 without specific exclusion criteria other than being apparently healthy. None were taking any medications known to affect heart rate or blood pressure. The investigation did not

specifically target children or adults of specific body composition phenotypes, or of any particular fitness level.

EXPERIMENTAL DESIGN

Participants reported to the laboratory in the morning for a single day of testing, 3 h postprandial fasting as a minimum, and rested quietly for at least 15 min in the supine position before measurement. Adult participants were asked to refrain from vigorous activity and from alcohol or caffeine intake over the day before the protocol. The study was conducted with room temperature ranging from 22 to 25 °C.

The sequence of measures was as follows: (1) total-body scans performed by dual-energy x-ray absorptiometry, (2) right brachial artery oscillometry, (3) right carotid artery stiffness measurement by means of echotracking, (4) contralateral brachial artery oscillometry, (5) arterial stiffness by applanation tonometry, (6) peak oxygen uptake exercise testing, and (7) recovery cardiovascular measurements (2-5) within 10 min immediately following a 3 min recovery protocol. This time point was chosen because previous research has shown that exercise substantially alters arterial stiffness 10 min following cessation of exercise [359, 389].

ANTHROPOMETRY

Standing and sitting height were measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg on a scale with an attached stadiometer (model 770, Seca; Hamburg, Deutschland) wearing minimal clothing and no shoes. Leg length in children was calculated by subtracting sitting height from standing height.

MATURITY

Maturity offset, that is, time before or after peak height velocity, was predicted in children 9-10 years of age with the equation of Mirwald et al. [106] using the following variables: leg length, sitting height, age, weight, and height.

DUAL-ENERGY X-RAY ABSORPTIOMETRY

Total-body scans were performed by dual-energy x-ray absorptiometry and analyzed using an extended analysis program for body composition (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, Waltham, Massachusetts, USA) to determine whole body fat mass and lean soft tissue. The same technician positioned the subjects, performed the scans and completed the scan analysis according to the operator's manual using the standard analysis protocol. Quality control with spine phantom was made every morning, and with step phantom every week.

BRACHIAL BLOOD PRESSURE

The right and left brachial SBP and DBP were measured following at least 15 minutes with the participants in the supine position using an automated oscillometric cuff (HEM-907-E, Omron, Tokyo, Japan). Two measurements were taken and if these values deviated by >5 mmHg, a third measurement was performed. The average of the two closest values was used. The mean arterial pressure (MAP) was calculated with the formula [DBP + 1/3 (SBP-DBP)].

RIGHT CAROTID ARTERY STIFFNESS INDICES AND BLOOD PRESSURE BY VASCULAR ULTRASOUND

The right carotid artery stiffness measurements were conducted with the patient in the supine position after at least a 15 min resting period before and within 10 min immediately following cessation of exercise. We used a ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy) with Quality Arterial Stiffness technology, based on radio frequency signal in a common carotid artery segment ~1 cm before the bifurcation. This software used a complex algorithm that could process all data coming from the arterial wall as radiofrequency signals, automatically measuring the modification of the arterial diameter between the systolic and diastolic phases. During imaging of the common carotid artery, the examiner obtained real-time feedback on measurement to optimize the probe position to have best scan plane with respect to the distension and diameter. Theoretically, carotid diameter waveforms were assessed by means of ultrasound and converted to carotid pressure waveforms using an empirically derived exponential

relationship between pressure and arterial cross-section. The derived right carotid pressure waveform was calibrated to right brachial end diastolic and mean arterial pressure by iteratively changing the wall rigidity coefficient. This allows the calculation of carotid stiffness indices: PWV (m/s), distensibility coefficient (1/KPa), compliance coefficient (mm^2/kPa), stiffness index α and β. Specifically, local carotid (LC) PWV was calculated from the following equation: $PWV = \frac{1}{\sqrt{\rho \cdot DC}} = \sqrt{\frac{D^2 \cdot \Delta PP}{\rho \cdot (2 \cdot D \cdot \Delta D + \Delta D^2)}}$ where, D: diastolic diameter; ΔD: change of diameter in systole; DC: distensibility coefficient; ΔPP: local pulse pressure; ρ: blood density. PWV is a functional parameter directly affected by arterial wall stiffness. DC is the fractional change in cross-sectional area relative to the change in arterial pressure. DC was calculated as: $DC = \frac{\Delta A}{A \cdot \Delta PP} = \frac{2 \cdot D \cdot \Delta D + \Delta D^2}{D^2 \cdot \Delta PP}$ where A: diastolic area; ΔA: change of area in systole. Compliance Coefficient (CC) was calculated as: $CC = \frac{\Delta A}{\Delta PP} = \frac{\pi \cdot (2 \cdot D \cdot \Delta D + \Delta D^2)}{4 \cdot \Delta PP}$; Stiffness index α was expressed as: $\alpha = \frac{A \cdot \ln\left(\frac{Ps}{P_d}\right)}{\Delta A} = \frac{D^2 \cdot \ln\left(\frac{Ps}{P_d}\right)}{2 \cdot D \cdot \Delta D + \Delta D^2}$ and stiffness index β was expressed as: $\beta = \frac{D \cdot \ln\left(\frac{Ps}{P_d}\right)}{\Delta D}$, where P_s and P_d are carotid systolic and diastolic pressure respectively.

The coefficients of variation for repeated measurements in our laboratory for LC PWV, DC, CC, stiffness index α and β, carotid SBP and DBP are: 1.89%, 0%, 2.87%, 3.70%, 3.18%, 2.15% and 0%, respectively.

CONTRALATERAL PULSE WAVE VELOCITY AND CAROTID BLOOD PRESSURE BY APPLANATION TONOMETRY

PWV was measured by applanation tonometry immediately after ultrasound imaging. A single operator located the carotid, femoral, radial and distal posterial tibial arteries on the left side of the body and marked the point for capturing the corresponding pressure curves with two specific pressure sensitive transducers. The distance between the carotid and femoral, radial and distal posterial tibial arteries was measured directly and entered into the Complior Analyse software (ALAM Medical, Paris, France). Left brachial blood pressure was measured and entered into the software, and then signal acquisition was launched. The carotid sensor, with the help of its specific holder, was positioned and a manually held sensor was placed on the femoral artery and the distal sensor was place on

the distal posterial tibial artery. Ten quality carotid pulse waveforms, and simultaneous carotid and femoral, radial and distal posterial tibial pressure curves were recorded. The time delay (aortic transit time) between the two pulse waveforms was calculated automatically according to the intersecting tangent algorithm [380]. The distance traveled by the pulse waveforms was measured between the two recording sites directly on the body surface and was corrected automatically according to the equation "0.8*direct distance" subtracting the manubrium-to-carotid distance as recommended [381]. PWV was then calculated using measurements of transit time and distance traveled by the pulse wave between the two recording sites. Values obtained from the carotid to femoral artery (CF), carotid to radial artery (CR) and carotid to distal posterior tibial artery (CD) were taken as indices of central/aortic, upper and lower limb arterial stiffness, respectively. The quality of the PWV records was immediately evaluated by a second observer with considerable experience in this methodology. Whenever a continuous decrease before the sharp systolic upstroke was not clearly seen or tolerance was above 5ms, a second measure was taken.

Carotid SBP from the Complior Analyser was obtained from left carotid traces acquired during the PWV assessment. The waveforms were averaged and the mean values were extracted from 15 s window of acquisition. The carotid waveforms were calibrated with left brachial MAP, measured immediately before the acquisition.

The coefficients of variation for repeated measurements in our laboratory for CF, CR and CD PWV, central/aortic SBP and DBP of young adult participants are: 2.95%, 9.10% and 4.11% and 2.45% and 0%, respectively.

MAXIMAL EXERCISE TEST

Peak oxygen capacity was determined using an individualized protocol on a motorized treadmill to exhaustion. The protocol started with a warm-up during 3 min in children and 5 min in adults, followed by 1 mph increments every 2 min for 4 min, after which 2.5% grade increments were added every minute until exhaustion. The speed of the treadmill in stage 1 was selected individually based on the participant's level of mobility and stride length. The protocol ended with a 1 min active recovery plus 2 min of passive recovery in the sitting position. Children had a treadmill training session before the test day.

Inspired and expired gases were continuously analyzed, breath-by-breath, through a portable gas analyzer (K4b^2, Cosmed, Rome, Italy). Before each test, the O_2 and CO_2 analyzers were calibrated using ambient air and standard calibration gases of known concentration (16.7% O_2 and 5.7% CO_2). The calibration of the turbine flowmeter of the K4b^2 was performed using a 3-l syringe (Quinton Instruments, Seattle, Wash., USA) according to the manufacturer's instructions. Heart rate (HR) was continuously monitored (Polar Electro Oy, Finland). The participants did not carry the gas analyser. Data were evaluated in 20-second averages, and VO2peak was defined as the highest 20-second value attained in the last minute of effort provided 2 of the following criteria were met: (1) Attaining ~90% of predicted maximal heart rate (220-age); (2) Plateau in VO2 with an increase in workload (<2.0 $mL.kg^{-1}.min^{-1}$); (3) Rating of perceived exertion of ≥ 8 for children (1-10) or ≥ 18 for adults (6-20) and; (4) Respiratory exchange ratio of ≥ 1.05 for children and ≥ 1.1 for adults; 5) subjective judgment by the observer that the participant could no longer continue, even after encouragement.

STATISTICAL ANALYSES

All values were expressed as mean ± standard error of the mean. Normality was verified using the Shapiro-Wilk test in each variable. Baseline group characteristics were compared with one-way ANOVA. Repeated measures analysis of variance was used to test for possible exercise and group interaction effects in non-adjusted and ΔMAP-, ΔPP- or height- adjusted arterial stiffness indices. Additionally, repeated measures ANOVA was used test for exercise and ΔMAP (small/high), ΔPP (small/high) or height (small/high) interaction effects when found significant in respective adjusted analysis. ΔMAP and ΔPP were calculated as: [(value at recovery – value at rest) / value at rest]. Statistical significance level was set at $p<0.05$ for all tests. The statistical analyses were computed and analyzed by a certified researcher using the SPSS Statistics 22.0 (IBM Corp. Armonk, NY USA).

RESULTS

BODY COMPOSITION AND ANTHROPOMETRICS

Children and adults had similar total body fat (24.33±1.15% vs. 21.62±0.97%), total lean soft tissue (72.35±1.10% vs. 74.61±0.95%) and abdominal fat (21.14±1.43% vs.21.060±1.04%) ($p>0.05$), but differed in height (127.59±1.87 vs. 169.81±1.34 cm) ($p<0.05$). All children were pre-pubertal (-1.87±0.38 years).

HEART RATE AND OXYGEN CONSUMPTION

Adults reached a higher percentage of predicted maximal HR (91.45±0.76% vs 95.19±0.66%) and respiratory exchange ratio (1.11±0.01 vs. 1.15±0.01) during the maximal exercise test ($p<0.05$) but the aerobic aptitude was similar between age groups (46.92±1.57 $ml.kg^{-1}.min^{-1}$ vs. 50.22±1.26 $ml.kg^{-1}.min^{-1}$) ($p>0.05$). Children had a faster HR recovery by the 3rd min after maximal effort than adults (74.34±0.03% vs. 55.29±0.017%) ($p<0.05$).

TABLE 16: HEIGHT ADJUSTED CAROTID AND BRACHIAL BLOOD PRESSURES AT REST AND AFTER A SINGLE BOUT OF MAXIMAL EXERCISE IN CHILDREN AND ADULTS.

		Children				Adults			
		Rest		Recovery		Rest		Recovery	
Right brachial SBP	(mmHg)	115.97 ± 2.91 [a]		127.39 ± 3.73 [a]		103.73 ± 2.18		110.30 ± 2.79	
Right brachial DBP	(mmHg)	58.43 ± 2.81		62.23 ± 2.81		63.57 ± 2.10		64.73 ± 2.10	
Right brachial MAP	(mmHg)	77.35 ± 3.01		78.50 ± 3.05		78.53 ± 1.58		78.74 ± 1.59	
Right carotid SBP	(mmHg)	112.10 ± 3.69 [a]		129.30 ± 4.60 [a]		94.99 ± 2.27		108.11 ± 2.82	
Right carotid DBP	(mmHg)	58.71 ± 3.24		60.75 ± 3.06		63.63 ± 2.23		66.30 ± 2.11	
Right carotid PP [b, c]	(mmHg)	52.94 ± 3.72 [a]		69.16 ± 5.26 [a]		31.32 ± 2.33		40.93 ± 3.29	
Left brachial SBP	(mmHg)	120.55 ± 2.94 [a]		120.87 ± 3.40 [a]		102.68 ± 1.98		107.24 ± 2.28	
Left brachial DBP	(mmHg)	61.76 ± 3.01		62.28 ± 3.03		63.52 ± 2.02		63.04 ± 2.04	
Left brachial MAP	(mmHg)	81.07 ± 2.64		82.12 ± 2.85		77.06 ± 1.67		77.81 ± 1.80	
Left carotid SBP	(mmHg)	110.61 ± 4.46		109.26 ± 5.05		104.54 ± 2.29		108.85 ± 2.60	
Left carotid DBP	(mmHg)	61.82 ± 3.46		61.96 ± 3.48		65.35 ± 1.96		63.94 ± 1.97	
Left carotid PP	(mmHg)	50.03 ± 5.47		47.92 ± 6.13		38.56 ± 2.86		44.60 ± 3.20	

Values are estimated marginal means and standard error of the mean. Statistical significance level was set at $p<0.05$. Covariates appearing in the model are evaluated at the following values: 152.70 to 158.49 cm. Legend: [a] Different from adults; [b] main exercise effect; [c] main exercise*group interaction effect; [d] main exercise*height interaction effect.

BRACHIAL BLOOD PRESSURE

Children had lower brachial SBP at rest and during recovery ($p<0.05$) (FIGURE 16). Brachial SBP increased (right/left: $\eta^2=0.456/\eta^2=0.108/$) following exercise along with right brachial DBP ($\eta^2=0.097$). Exercise*group interaction effects were found in right brachial DBP ($\eta^2=0.100$) as the pressure increased in children following exercise compared to no change in adults. When measures were adjusted for participant's height, SBP at rest and after exercise were higher in children ($p<0.05$) (TABLE 16). No significant exercise or group interaction effects were found in the adjusted values.

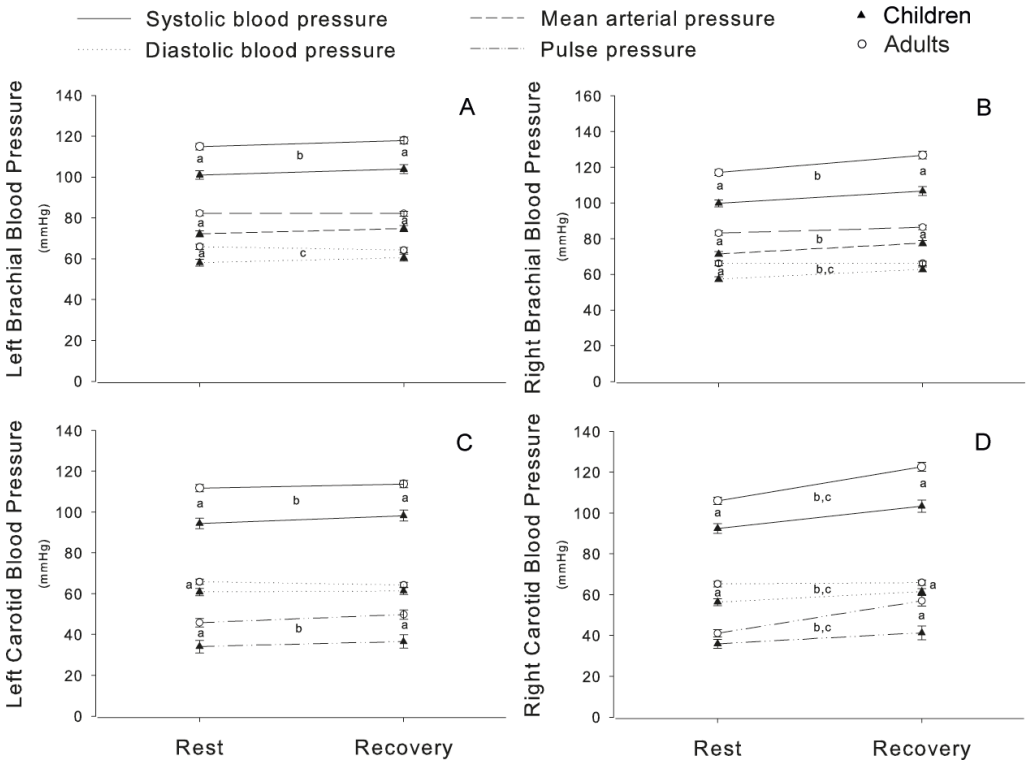

FIGURE 16: CENTRAL AND PERIPHERAL BLOOD PRESSURE MEASURES AT REST AND AT RECOVERY AFTER MAXIMAL EXERCISE IN CHILDREN AND ADULTS.

Left carotid blood pressure was obtained applanation tonometry whereas right carotid blood pressured was obtained by echotracking. Values are expressed as mean and standard error of the mean. Statistical significance level was set at $p<0.05$. Legend: [a] Different from adults; [b] main exercise effect; [c] main exercise*group interaction effect.

CAROTID BLOOD PRESSURE

Children had lower carotid blood pressure at rest and at recovery ($p<0.05$) (FIGURE 16). Carotid SBP (right/left: $\eta^2=0.617$/$\eta^2=0.075$) and PP (right/left: $\eta^2=0.394$/$\eta^2=0.066$) increased after exercise ($p<0.05$) regardless of the measurement technique. Significant exercise*group interaction effects were found in right carotid SBP ($\eta^2=0.068$) and DBP ($\eta^2=0.067$) and PP ($\eta^2=0.136$) as maximum pressure increased more in adults, together with no change in DBP. When measures were adjusted for participant's height, right carotid SBP and PP were higher in children at rest and during recovery ($p<0.05$) (TABLE 16). Right PP significantly increased after exercise ($\eta^2=0.069$) in particular in taller participants ($\eta^2=0.111$; $p<0.05$).

CAROTID ARTERY STIFFNESS INDICES

At both time points, children had higher CC and DC, and a smaller carotid diameter coupled with lower stiffness index α and β, even when adjusted for ΔMAP or carotid ΔPP ($p<0.05$) (FIGURE 17; TABLE 17; TABLE 18).

TABLE 17: ΔMAP ADJUSTED ARTERIAL STIFFNESS INDICES AT REST AND AFTER A SINGLE BOUT OF MAXIMAL EXERCISE IN CHILDREN AND ADULTS.

		Children				Adults			
		Rest		Recovery		Rest		Recovery	
Local Carotid PWV [b, c]	(m/s)	3.912 ± 0.115 [a]		4.160 ± 0.124 [a]		4.791 ± 0.096		5.374 ± 0.104	
Central/Aortic PWV	(m/s)	5.161 ± 0.289 [a]		5.560 ± 0.321 [a]		6.963 ± 0.218		6.791 ± 0.243	
Upper Limb PWV	(m/s)	10.367 ± 0.398		9.816 ± 0.493		9.368 ± 0.301		8.987 ± 0.373	
Lower Limb PWV [b, d]	(m/s)	6.727 ± 0.233 [a]		6.103 ± 0.225 [a]		7.947 ± 0.176		7.467 ± 0.170	
Compliance Coefficient [b, c]	(mm^2/kPa)	1.612 ± 0.078 [a]		1.466 ± 0.075 [a]		1.278 ± 0.063		0.971 ± 0.060	
Distensibility Coefficient [b]	(1/kPa)	0.071 ± 0.004 [a]		0.060 ± 0.003 [a]		0.042 ± 0.003		0.034 ± 0.002	
Stiffness Index α [b, d]		2.126 ± 0.107 [a]		2.080 ± 0.112 [a]		2.684 ± 0.088		2.810 ± 0.091	
Stiffness Index β [b, d]		4.520 ± 0.219 [a]		4.427 ± 0.225 [a]		5.647 ± 0.179		5.929 ± 0.184	
Carotid Diameter [a]	(mm)	5.70 ± 0.09 [a]		5.58 ± 0.10 [a]		6.201 ± 0.07		6.03 ± 0.07	
Distension	(mm)	0.75 ± 0.03		0.73 ± 0.04		0.67 ± 0.02		0.70 ± 0.03	

Local carotid PWV was obtained by echotracking whereas, central/aortic, upper and lower limb PWV were obtained by applanation tonometry. Values are estimated marginal means and standard error of the mean. Statistical significance level was set at $p<0.05$. Covariates appearing in the model are evaluated at the following values: 0.015 to 0.067. Legend: [a] Different from adults; [b] main exercise effect; [c] main exercise*group interaction effect; [d] main exercise*ΔMAP interaction effect.

Significant main exercise effects were found in non-adjusted and ΔMAP-adjusted CC (η^2=0.385; η^2=0.345), DC (η^2=0.299; η^2=0.267) and carotid diameter (η^2=0.201; η^2=0.249) as values decreased following exercise, but only for CC when adjusted for ΔPP (η^2=0.183). Maximal exercise induced increases in stiffness index α (η^2=0.065) and β (η^2=0.076) but only when values were adjusted for ΔMAP. Exercise*group interaction effects were found in CC (η^2=0.088) as it decreased more in adults. Significant exercise*ΔPP interaction effects (η^2=0.432) were found when adjusted to ΔPP. A deeper analysis showed that CC (η^2=0.80) and DC (η^2=0.07) decreased more in those with high ΔPP. Significant exercise*group, exercise*ΔMAP and exercise*ΔPP interaction effects were also found in stiffness index α (η^2=0.053; η^2=0.102; η^2=0.112) and β (η^2=0.059; η^2=0.115; η^2=0.135), as only adults, those with small ΔMAP (η^2=0.092; η^2=0.102) or high carotid ΔPP (η^2=0.102; η^2=0.0.112) had increased stiffness following exercise. When measures were adjusted for participant's height, children had lower CC at rest ($p<0.05$) (TABLE 19). There were no significant exercise, height or group interaction effects.

TABLE 18: ΔPP ADJUSTED CAROTID ELASTIC FUNCTION AT REST AND AFTER A SINGLE BOUT OF MAXIMAL EXERCISE IN CHILDREN AND ADULTS.

		Children				Adults			
		Rest		Recovery		Rest		Recovery	
Local Carotid PWV [b, c]	(m/s)	3.912 ± 0.115 [a]		4.160 ± 0.124 [a]		4.791 ± 0.096		5.374 ± 0.104	
Compliance Coefficient [b, d]	(mm^2/kPa)	1.549 ± 0.087 [a]		1.281 ± 0.069 [a]		1.252 ± 0.062		0.987 ± 0.049	
Distensibility Coefficient [c, d]	(1/kPa)	0.068 ± 0.004 [a]		0.053 ± 0.003 [a]		0.041 ± 0.003		0.035 ± 0.002	
Stiffness Index α [d]		2.162 ± 0.119 [a]		2.117 ± 0.128 [a]		2.708 ± 0.089		2.818 ± 0.096	
Stiffness Index β [d]		4.593 ± 0.242 [a]		4.519 ± 0.257 [a]		5.696 ± 0.181		5.940 ± 0.192	
Carotid Diameter [b]	(mm)	5.729 ± 0.087 [a]		5.606 ± 0.097 [a]		6.183 ± 0.065		6.016 ± 0.072	
Distension [d]	(mm)	0.75 ± 0.04		0.76 ± 0.04		0.66 ± 0.03		0.68 ± 0.03	

Values are estimated marginal means and standard error of the mean. Statistical significance level was set at $p<0.05$. Covariates appearing in the model are evaluated at the following values: 0.3162 to 0.3281. Legend: a Different from adults; b main exercise effect; c main exercise*group interaction effect; d main exercise*ΔPP interaction effect.

PULSE WAVE VELOCITY

Children had lower LC, CF and CD in non-adjusted and ΔMAP- adjusted PWV at rest and at recovery ($p<0.05$) (FIGURE 18; TABLE 17). Following exercise, stiffness increased in LC ($\eta^2=0.444$) and decreased in the lower limb ($\eta^2=0.115$) even when adjusted to ΔMAP ($\eta^2=0.363$; $\eta^2=0.276$). Exercise*group interaction effects were found in non-adjusted, ΔMAP- or ΔPP adjusted LC PWV ($\eta^2=0.125$; $\eta^2=0.113$; 0.031) meaning that LC PWV increased more in adults and that ΔMAP did not add to the explanation. However, LC PWV increased more in participants with high carotid ΔPP. Exercise*ΔMAP interaction effects were found in CD PWV ($\eta^2=0.111$) as only those with small ΔMAP had decreased CD PWV following exercise ($\eta^2=0.111$). When measures were adjusted for participant's height, only CR PWV was different between children and adults at rest and recovery ($p<0.05$) (TABLE 19). No significant exercise, height or group interaction effects were found.

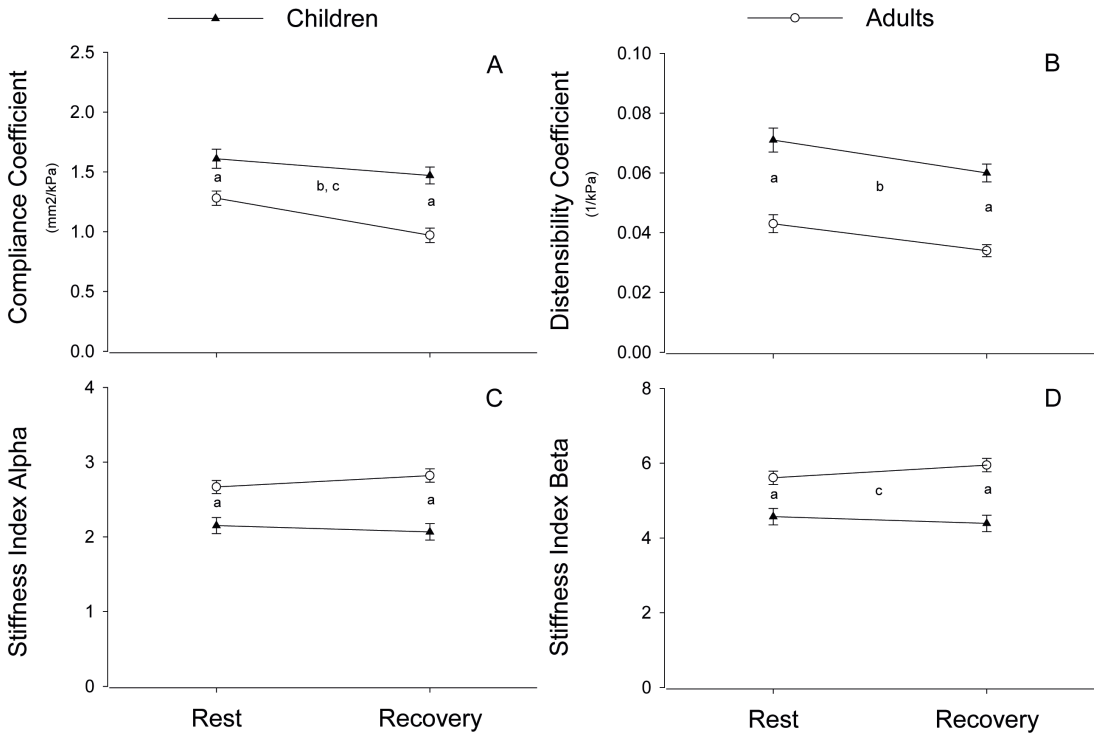

FIGURE 17: RIGHT CAROTID STIFFNESS INDICES AT REST AND AT RECOVERY AFTER MAXIMAL EXERCISE IN CHILDREN AND ADULTS.

Values are expressed as mean and standard error of the mean. Statistical significance level was set at $p<0.05$. Legend: [a] Different from adults; [b] main exercise effect; [c] main exercise*group interaction effect.

DISCUSSION

The key findings of this study were: a) carotid distending pressure increased immediately following exercise in children and adults; b) a single bout of maximal exercise increased carotid stiffness indices whilst decreasing stiffness in the exercised limb; c) the changes in stiffness may be related to the hemodynamic response; d) children and adults did not differ in vascular or hemodynamic responses at a comparable intensity level once adjusted to height; e) central/aortic PWV by applanation tonometry and local carotid PWV by echotracking should not be used interchangeably in the measurement of central stiffness and central hemodynamics.

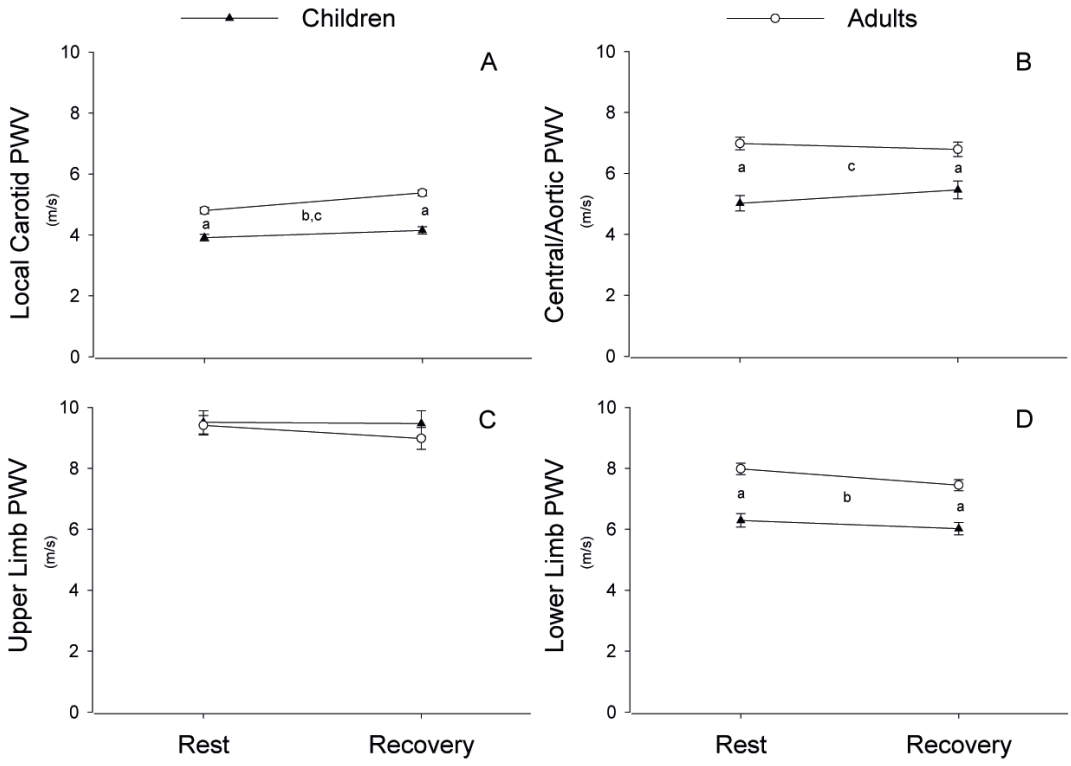

FIGURE 18: PWV AT REST AND AT RECOVERY AFTER MAXIMAL EXERCISE IN CHILDREN AND ADULTS.

Local carotid PWV was obtained by echotracking whereas, central/aortic, upper and lower limb PWV were obtained by applanation tonometry. Values are expressed as mean and standard error of the mean. Statistical significance level was set at $p<0.05$. Legend: [a] Different from adults; [b] main exercise effect; [c] main exercise*group interaction effect.

The physiologic response to acute exercise is complex and may be mechanistically linked to the increased arterial distensibility observed with chronic exercise training. This is the first study comparing local, aortic and peripheral stiffness indices, following acute maximal treadmill exercise in children and young adults and sheds light on our understanding of the exercise-mediated signals for arterial adaptations. Herein, we have evidenced in 2 distinct age groups that an acute bout of maximal exercise can alter arterial stiffness in vasculatures perfusing the brain and within the active muscle beds. Given that localized exercise training does not exert systemic effects on the vasculature [354], it is possible that there is a threshold for hemodynamic forces and circulatory factors that may only be reached when exercise involves large muscle groups [390]. In addition, our findings indicate that a lower stiffness and hemodynamic response to metabolic demands during exercise in children appear to reflect their smaller size which may explain why arterial stiffness gradually increases throughout childhood [1, 391].

TABLE 19: HEIGHT ADJUSTED ARTERIAL STIFFNESS INDICES AT REST AND AFTER A SINGLE BOUT OF MAXIMAL EXERCISE IN CHILDREN AND ADULTS.

		Children				Adults			
		Rest		Recovery		Rest		Recovery	
Local Carotid PWV	(m/s)	4.726 ± 0.191		5.051 ± 0.204		4.223 ± 0.140		4.752 ± 0.151	
Central/Aortic PWV	(m/s)	6.370 ± 0.438		6.852 ± 0.518		6.019 ± 0.329		5.798 ± 0.388	
Upper Limb PWV	(m/s)	11.092 ± 0.691 [a]		10.792 ± 0.786 [a]		8.251 ± 0.532		8.017 ± 0.605	
Lower Limb PWV	(m/s)	7.516 ± 0.390		6.682 ± 0.375		7.052 ± 0.307		6.942 ± 0.296	
Compliance Coefficient	(mm^2/kPa)	1.079 ± 0.134 [a]		0.946 ± 0.127		1.625 ± 0.093		1.309 ± 0.088	
Distensibility Coefficient	(1/kPa)	0.048 ± 0.007		0.039 ± 0.005		0.058 ± 0.005		0.049 ± 0.003	
Stiffness Index α		2.502 ± 0.202		2.323 ± 0.212		2.431 ± 0.145		2.647 ± 0.152	
Stiffness Index β		5.332 ± 0.413		4.995 ± 0.427		5.099 ± 0.296		5.546 ± 0.306	
Carotid Diameter	(mm)	5.93 ± 0.15		5.73 ± 0.17		5.99 ± 0.11		5.87 ± 0.12	
Distension	(mm)	0.80 ± 0.06		0.87 ± 0.07		0.63 ± 0.04		0.61 ± 0.05	

Local carotid PWV was obtained by echotracking whereas, central/aortic, upper and lower limb PWV were obtained by applanation tonometry. Values are estimated marginal means and standard error of the mean. Statistical significance level was set at $p<0.05$. Covariates appearing in the model are evaluated at the following values: 151.64 to 158.10 cm. Legend: [a] Different from adults; [b] main exercise effect; [c] main exercise*group interaction effect; [d] main exercise*ΔPP interaction effect.

CENTRAL AND PERIPHERAL HEMODYNAMIC RESPONSE

The carotid SBP in children and adults were similar to reference values reported in literature [392, 393] showing an increase in central blood pressure with age. However, our data also show that differences in height may be the major reason for these age differences. This finding is consistent with previous work [392].

Several studies have shown that an acute bout of aerobic exercise leads to an immediate drop in brachial artery [394, 395], central/aortic [361, 396, 397] and carotid blood pressure [398]. Interestingly, this pattern was found to be much less consistent in normotensive individuals [399, 400]. In our study, maximal exercise induced a 3.92% (adults) to 8.47% (children) increase in right brachial mean arterial pressure, and 14.98% (children) to 38.49% (adults) increase in carotid PP. The changes in PP were greater in adults due to greater increases in carotid SBP following exercise. The inconsistency in findings in post exercise hypotension between studies may be due in part to the intensity of the stimulus as hemodynamic after-effects of exercise apparently depend on the severity of the load [401]; to the duration of the effort and the health status of the study population as shorter bouts of exercise elicit inconsistent changes in arterial pressure in normotensive but not in hypertensive individuals [402] and; to the time of measure [401, 403]. DeVan et al. [387] have shown that although peripheral SBP remained constant following exercise, central SBP was significantly elevated above baseline levels immediately after exercise. Our data are consistent with these findings and those of Rossow et al. [366]. In addition, our results show that the response patterns of central and peripheral blood pressure to exercise are not age-dependent once the values were adjusted for body size.

CAROTID ARTERY STIFFNESS INDICES

Carotid stiffness indices at rest increased with age in accordance with previous studies [1, 391]. Stiffness in the carotid artery also increased following maximal exercise. Early post-exercise, the carotid artery decreased in diameter, consistent with vasoconstriction in both children (1.6%) and adults (3%). The mechanism of the post-exercise carotid vasoconstriction is not clear, but strenuous dynamic exercise is associated with high levels of sympathetic vasoconstrictor activity and plasma noradrenaline concentrations [404, 405], and the carotid artery smooth muscle is known to be innervated by sympathetic

efferents and to express α1 adrenergic receptors [406]. Thus sympathetically mediated vasoconstriction may have contributed. Alternatively, Studinger et al. [407] showed that at high work intensities the elevated arterial pressure induces a strong myogenic response in the carotid smooth muscle [408]. When exercise is terminated and the intraluminal distending pressure suddenly drops, the myogenic smooth muscle contraction is left unopposed, causing a transient vasoconstriction. Myogenic vasoconstriction results in baroreceptor unloading and consequent sympathetic excitation [407]. CC and DC in children and adults decreased following exercise as both coefficients are largely influenced by local pressure (denominator). Our data indicates that changes in CC and DC following maximal exercise were mediated primarily by local changes in distension pressure as shown by the significant exercise*ΔPP interaction effect found, and that the measurement of peripheral blood pressure during exercise recovery provides little insight into the central vascular burden associated with high-intensity dynamic exercise [366]. Moreover, there was no change in the stiffness index α and β after the exercise bout further supporting the importance of local pressure. Interestingly, when we controlled both indices for local and systemic distending pressures we witnessed an exercise*distending pressure interaction effect, meaning that changes in stiffness are related to changes in pressure.

Exercise induced tachycardia results in an overall shortening of the cardiac cycle, particularly in diastole [366] and this may shorten carotid vessel recoil during diastole [407]. When examining the denominator of the stiffness index α and β it can be seen that these indices are particularly influenced by distension. However, no significant changes in distension following exercise were seen (data not shown), thus it is our opinion that the increased HR following exercise did not affect the viscoelastic vascular properties as proposed by Studinger et al. [407].

PULSE WAVE VELOCITY

Our results show a considerable increase in resting PWV with age along the arterial tree, indicating an increase in arterial stiffness with age [326]. The CF PWV values in both groups were slightly higher than optimal values reported in literature [380, 392].

Maximal aerobic exercise bout triggered a 4%-7% decrease in stiffness in the arteries of the exercised limb in children and adults. The magnitude of the lower limb vascular

response to exercise is similar between modes of leg exercise such as treadmill and cycling [354, 359]. Contradictory to the carotid artery response to exercise discussed above, the decrease in stiffness in the lower limb is likely due to the vasodilatory response to the exercise, presumably transferring stress from the less extensible collagenous elements in the wall to the more elastic elastin fibers [409]. In addition, the mechanical compression of the arteries during muscular contraction reduces arterial stiffness of the exercised limb [410]. This may explain why there was a significant decrease in PWV in the exercised limb as compared to no significant change in large central/aortic arterial properties after a maximal exercise bout [354, 389, 411, 412].

Carotid and central/aortic PWV can be both considered measures of central stiffness, but our results show these arteries yield different values at rest and following exercise. LC PWV increased 6% to 12% following exercise in both groups whereas no significant change was observed in CF PWV. Although both the carotid artery and the aorta are classified as elastic vessels, the ultrastructure of the carotid artery is more like the abdominal aorta than the ascending aorta. Discrepancies between carotid and aortic arteries could also result from inaccurate measurements. CF PWV is generally considered the gold standard for the direct measurement of aortic stiffness [413]. However, the distance between the carotid and femoral sites is measured manually and may differ from the true length of the arterial pathway because of anatomic particularities. An identical method would be difficult to apply to the carotid artery because of the short vessel path (10 to 15 cm) and subsequent short time lag (5 to 15 ms) [414]. Therefore, LC is determined cross-sectionally from local changes in pressure and artery diameter. PWV may also be influenced by heart rate [415] although only heart rates above 120 bpm appear to affect PWV measures [416].

Considering the high interdependence of arterial stiffness and arterial pressure [409, 417], it would be understandable to suspect that disparate blood pressure responses between groups confounded the results. There were no main exercise effects in left brachial mean arterial pressure following exercise, however the exercise*ΔMAP interaction effect in ΔMAP adjusted analysis showed that those participants with small ΔMAP decreased lower limb PWV more following exercise, suggesting that the mechanisms responsible for the alteration in PWV may be related at least in part to the hemodynamic response [418]. Other studies found reductions in stiffness after acute aerobic exercise [328, 419], and

increases in stiffness after acute resistance exercise [387], with no change in brachial mean blood pressure. However, they did not go further on adjusting the analysis for changes in distending pressure and used longer time of recovery (20-60 min following exercise).

The noninvasive assessment of PWV is critically dependent on the measurement of the travel distance of the arterial pulse wave [420]. Travel distance is proportional to body height [421]. Wang et al [422] recognized the problem and adjusted the hazard ratios expressing the risk of death related to a pulse wave reflection index for body height in adults. Doyon et al. [1] observed in children that growth abnormalities need to be considered in the assessment of arterial distensibility given that children who are very short or tall for age might be assessed more accurately by height-normalized reference values. Although biologically it is possible that age-related changes in resting arterial stiffness noted in this study reflect the precedents of vascular ageing, since it may be affected by structural factors like the composition of the arterial wall including the contents of the extracellular matrix [423], an alternative or additional mechanism underlying the observed changes from childhood to adulthood might be adaptive remodeling of the vessel walls in response to physiological developmental changes in body dimensions or/and blood pressure [1]. Whereas our data cannot conclusively answer the question whether vascular aging or changing body dimensions are more relevant for the vascular differences observed, we showed that there were no significant differences in PWV at rest or following exercise when measures were adjusted to the height of the participants.

LIMITATIONS

The estimates of normal MAP in the literature for adults may not be valid for young children or for altered HR. Vasodilation could potentially cause an overestimation of MAP for any given cardiac output, and at higher HR, MAP is more closely approximated by the arithmetic average of systolic and diastolic pressure because the shape of the arterial pulse changes as the period of diastole shortens more than does systole [424]. However, this method provides a valid estimation of MAP over a wide range of exercise intensities in adults [425] and it this should not have influenced our conclusion regarding the relative changes in children.

Menstrual cycle variation was not controlled in adult female subjects. We recognize that while it has been reported that elastic properties of central arteries do not fluctuate significantly with the phases of the menstrual cycle [426-429], and that artery wall properties are not affected by oral contraceptive use [430], this is not a universal finding [429]. In our study, both male and female participants had similar PWV at rest and at recovery irrespective of the segment measured ($p>0.05$; data not shown), suggesting that there were no sex differences in the arterial response to acute maximal exercise. These findings are supported by previous reports suggesting that men and women have similar arterial responses to acute perturbations [354, 386, 410].

Some children were not tested for vascular assessment in the morning. Although they were asked to respect the same pre-requisites this may have been an unavoidable source of bias. However, data obtained from a postprandial and post-exercise study show that there were no consistent differences in PWV under fasting or post-prandially in adults [431]

CONCLUSION

An acute bout of maximal exercise can alter arterial stiffness and hemodynamics in vasculatures perfusing the brain and within the active muscle beds. In addition, our findings indicate that arterial stiffness and hemodynamic response to metabolic demands during exercise in children simply reflects their smaller body size and may not indicate a particular physiologic difference compared to adults.

CHAPTER 3

WHEN TO PREVENT CARDIOVASCULAR DISEASE?

1. BACKGROUND

We could prevent 90% of heart attacks. Such a claim would have seemed outrageous in the past but the identification of risk factors that predict the probability of coronary heart disease stimulated hope that modification of these risk factors would reduce cardiac events [432]. This claim is never before been so achievable. The absence of established risk factors at 50 years of age is associated with very low lifetime risk for CVD and markedly longer survival [433]. Compared with participants with 2 or more major risk factors, those with optimal levels had substantially lower lifetime risks (5.2% versus 68.9% in men, 8.2% versus 50.2% in women) and markedly longer median survivals (>39 versus 28 years in men, >39 versus 31 years in women). These results should promote efforts aimed at preventing development of risk factors in young individuals.

THE CHILDHOOD ORIGIN OF ATHEROSCLEROSIS

Minimal eccentric thickening to complete occlusion of one or more of the main coronary branches were found in 77.3% of young American soldiers killed in Korea war [434]. Vietnam war casualties had a similar prevalence [435]. The natural history of atherosclerosis from childhood fatty streaks to clinically significant fibrous plaques during young adulthood has since then been established [436]. Fibrous plaques then undergo a variety of changes (hemorrhage, rupture, thrombosis) that lead to obstruction and clinically manifest coronary heart disease.

As the natural history of atherosclerosis was appreciated and the effects of the risk factors in adult CVD were demonstrated, investigators began to examine the prevalence of risk factors for adult CVD in children and adolescents in widely separated communities [437-439]. Dyslipidemia, high blood pressure, smoking, and obesity were already present. Increased ponderosity was associated with adverse changes in lipids and lipoproteins and blood pressure concluding that there is a secular trend toward increased ponderosity in children, associated with worsening cardiovascular risk, particularly with regard to lipoproteins [440, 441]. Both high-density and low-density lipoprotein cholesterol levels in grandchildren were strong predictors of coronary heart disease in grandfathers [442]. In addition, serum lipids [443], blood pressure [444], and fatness [445] tracked through childhood and into young adulthood.

LINKING MODIFIABLE YOUTH RISK FACTORS WITH SURROGATE MARKERS OF SUCLINICAL ATHEROSCLEROSIS

Evaluation of arterial structure and stiffness and its predictors may help identify asymptomatic individuals at risk. Whether and to what extent childhood traditional cardiovascular risk factors and their cumulative burden from childhood to adulthood are associated with arterial structure and stiffness measured in young adulthood has not been clearly established. From a public health perspective, it would be important to determine whether decrease the cardiovascular risk factors in adult age can reverse the adverse effects of CVD risk factors in childhood or whether childhood risk factors increases cardiovascular risk independently of adult risk factors.

ELEVATED BLOOD PRESSURE

Systolic blood pressure in childhood is associated with arterial structure and stiffness in adulthood [4, 89, 222, 446, 447]. Importantly, the prospective association observed for SBP and cIMT tended to be stronger than the cross-sectional association [4]. Associations with youth pulse [448] and diastolic [4] pressures have also been shown.

Data from The Amsterdam Growth and Health Longitudinal Study showed that subjects with stiffer arteries had on average and throughout the whole longitudinal period 4.7-5.7 mmHg higher mean arterial, diastolic, and systolic pressures from age 13 to 36 years compared with those with less stiff arteries, independently of fatness levels. Importantly, these differences were not constant over time, and although being already present during adolescence were further amplified from this age onward and more strongly so in subjects with stiffer arteries than with less stiff arteries [449]. Results using data from 4 large prospective cohorts that have followed up individuals from childhood to adulthood suggested that SBP was associated with cIMT since the age of 6 [450]. In addition, the authors conclude that the age of risk factor assessment is an important consideration in the identification of children who will be at increased risk of having subclinical atherosclerosis in young adulthood. Risk factors measured before the age of 9 years have only weak or non-significant associations with cIMT measured more than 20 years later, whereas analysis among subjects 9 to 18 years of age showed significant associations between childhood risk exposure and increased adult cIMT [450].

OVERWEIGHT AND OBESITY

Previous evidence have shown vascular damage is suggested to have origins in childhood body fatness and body fat distribution independently of other risk factors [4, 117, 222, 451-454], but it was unclear whether this was a direct effect of childhood fatness or an indirect effect arising from tracking of obesity from childhood to adulthood. To clarify this topic, Huynh et al. [455] examined the relationships of body size or fatness in childhood (7–15 years) and 20 years later in adulthood (26–36 years) with adult cIMT and large artery stiffness on a sizable population-based sample of Australians. Large artery stiffness was found to depend primarily on current fatness and was greatest for those who were normal weight in childhood but obese as adults, whereas cIMT was positively associated with body size or fatness in both childhood and adulthood. This suggests that accumulation of cIMT occurs slowly from childhood to adulthood, whereas large artery stiffness is more dynamic and dependent on current fatness and magnitude of fatness gain between childhood and adulthood.

Data from the Amsterdam Growth and Health Longitudinal Study indicates that BMI levels differed significantly from those with less stiff arteries already at age 15 years, and this difference was approximately twice as much at age 36 years [449], whereas Juonala et al. [450] suggest that differences in BMI between those with high and not high cIMT in adulthood can be identified from the age of 9 years. Although an effect of youth fatness on adult preclinical markers has been consistent, debate remained as to the independence of the youth fatness effect over adult fatness [456].

To test whether the association between childhood fatness and cardiovascular risk persists when overweight or obese children become non-obese as adults was the main purpose of Juonala et al. [457]. The authors showed that those overweight in youth were at 30% increased risk of having high cIMT in adulthood but, among those who were able to become non-obese adults, the risk was reduced to levels observed for those who were never overweight or obese. Although the observational nature of this study precludes making clinical recommendations, reducing BMI in children and adolescents who are overweight or obese could reduce their cardiovascular risk. If this hypothesis is correct, primary care physicians should not take the pessimistic view that once childhood obesity is established, cardiovascular risk is also determined but should recognize that

cardiovascular risk may be substantially reduced if childhood obesity is successfully treated. Nevertheless it is necessary to consider that child fatness is the strongest predictor of adult fatness [458] and that, once obesity is established, it is very difficult to reverse.

Not all obese adults have cardiometabolic abnormalities. It is unknown whether this is true in children and, if so, whether children who have metabolically healthy overweight/obesity will also have favorable cardiometabolic profiles in adulthood. These aspects were examined by Li et al. [459] in 1098 individuals who participated as both children (aged 5-17 years) and adults (aged 24-43 years) in the Bogalusa Heart Study. Forty-six children (4.2%) were metabolically healthy overweight/obesity, and they were more likely to retain this status in adulthood compared with children in other categories. Despite markedly increased obesity in childhood and in adulthood, these same metabolically healthy overweight/obesity children and adults showed a cardiometabolic profile generally comparable to that of non-overweight/obese children. Moreover, there was no difference in cIMT in adulthood between metabolically healthy overweight/obesity children and non-overweight/obese children. Further, cIMT in adulthood was lower in metabolically healthy overweight/obesity children than in metabolically abnormal, overweight/obese children. Whether metabolically healthy overweight/obesity children should be targeted with weight-loss interventions remains unknown.

PHYSICAL INACTIVITY AND LOW CARDIORESPIRATORY FITNESS

Not only current but also adolescent levels of CRF were inversely and independently associated with cIMT but not with arterial distensibility and compliance in the Amsterdam Growth and Health Longitudinal Study [210]. The relationships between changes in CRF [from adolescence (13–16 yr) to adulthood, and from young adulthood (21–32 years-old) to adulthood] and cIMT and stiffness of the carotid, femoral, and brachial arteries, in men and women aged 36 were later investigated [243]. The authors found that longitudinal changes in CRF and reported physical activity levels from adolescence to age 36 were not associated with cIMT but changes in CRF were inversely associated with carotid stiffness, a relationship in part dependent on, and possibly mediated by changes in other risk factors. The same group examined whether lifetime habitual physical activities of different intensities carry the same protective effect on arterial stiffness and to what extent any such

effect is mediated by other biological cardiovascular risk factors [460]. Subjects with lower distensibility and compliance at the age of 36 years (as assessed by different local stiffness estimates) spent significantly less time in vigorous but not in light-to-moderate–intensity physical activity between adolescence and young adulthood, supporting the view of a favorable impact of vigorous physical activity on carotid arterial stiffness. This favorable impact was explained by the beneficial vigorous physical activity related changes in other CVD risk factors. Finally, despite considerable decreases in time spent in vigorous physical activity during adolescence, compared with subjects with higher distensibility and compliance, those with stiffer carotid arteries were characterized by steeper decreases in time spent in vigorous physical activity during late adolescence and consistently less times in these physical activities thereafter, up to the age of 36 years.

Using data from the Special Turku Coronary Risk Factor Intervention Project, Pahkala et al. [82] showed that leisure-time physical activity was directly inversely associated with aortic IMT (P=0.011) after adjustment for age, sex, BMI, high-density lipoprotein/total cholesterol, SBP, and C-reactive protein and regarding FMD brachial artery diameter. Sedentary adolescents who increased their leisure-time physical activity from <5 to >5 MET $h.wk^{-1}$ between 13 and 17 years of age decreased progression of aortic IMT compared with adolescents who remained sedentary. Importantly, aortic IMT progression was attenuated in persistently active adolescents compared with those who became sedentary (P=0.0072). Juonala et al. [461] were the first to study the association between childhood risk factors and 6-year change in cIMT in adulthood. Childhood physical activity was inversely associated with adulthood cIMT progression even when taking into account the effects of adulthood risk variables.

Collectively, these data show that even modest increases in physical activity can have meaningful effects on preclinical markers and that strategies to maintain or increase physical activity from adolescence might be critical.

MULTIPLE RISK FACTORS

The number of risk factors identified in childhood is inversely associated with the extent of preclinical CVD, supporting the concept that multiple youth risk factors have a synergistic effect on preclinical CVD later in life [4, 22, 285, 447, 462, 463]. Moreover, these data

underscore the importance of prevention strategies, which focus on multiple risk factors, rather than a specific risk factor [456].

In 2010, the American Heart Association set its 2020 Strategic Impact Goal to improve the cardiovascular health of all Americans by 20% while reducing deaths from CVD and stroke by 20% [464] making a paradigm shift. It incorporated not only the avoidance of risk factors for CVD but also emphasized low-risk or ideal cardiovascular health profiles incorporating 7 modifiable CVD risk factors (smoking, BMI, physical activity, diet, blood pressure, total cholesterol, fasting glucose). The American Heart Association has defined ideal cardiovascular health metrics as ideal, intermediate, and poor. The applicability of this concept to a cohort of children and its relationship with cardiometabolic outcomes in adulthood has been reported by Laitinen et al. [465]. The authors observed a difference of 0.067 mm in adult cIMT between ideal child cardiovascular health index groups 1 and 6. This corresponds to 11.8 years, which means that participants with only 1 ideal health metric were almost 12 years older in terms of vascular age than those with 6 metrics.

2. Purpose

Because long-term clinical trials to test the potential benefit of exposure modification in youth on development of CVD in adulthood are not possible, cohort studies with measurements across the life-course provide the best opportunity to understand the potential effects of risk factors on an individual's path toward CVD. Combining this advantage with non-invasive imaging, cohort studies are able to provide data directly linking youth risk factors with preclinical markers of cardiovascular health in adulthood.

The purpose of the current chapter of the dissertation was to overview and provide recent findings linking modifiable youth risk factors to preclinical markers of CVD in adulthood, in particular blood pressure. The research carried out on this subject as part of the present doctoral research program resulted in the following manuscript, and communications (oral/poster) as co- author:

PEER-REVIEWED ARTICLES THAT ARE RELATED TO cIMT

Melo, X., Santos, DA., Santa-Clara, MH., Ornelas, R., Sardinha, LB. *Contributions Of Pulse Pressure In Adolescence And Adulthood To Vascular Health Of Young Adults*. Non-submitted.

ABSTRACTS THAT ARE RELATED TO THE LIFE-COUSE ANALYSIS OF THE DETERMINANTS OF SUBCLINICAL ATHEROSCLEROSIS

Ornelas, R., **Melo, X**., Santos, DA., Santa-Clara, MH., Sardinha, LB. *Contributions Of Pulse Pressure In Adolescence And Adulthood To Vascular Health Of Young Adults*. In: 8th European Youth Heart Study Scientific Symposium, 2015. Oslo. Abstract Book Of The 8th European Youth Heart Study Scientific Symposium. 2015. (Poster Session)

3. CONTRIBUTIONS OF PULSE PRESSURE IN ADOLESCENCE AND ADULTHOOD TO VASCULAR HEALTH OF YOUNG ADULTS

Melo, X., Santos, DA., Santa-Clara, MH., Ornelas, R., Sardinha, LB. *Contributions Of Pulse Pressure In Adolescence And Adulthood To Vascular Health Of Young Adults*. Non-submitted.

ABSTRACT

BACKGROUND: The pulsatile component of blood pressure (PP) is the consequence of intermittent ventricular ejection from the heart. PP is influenced by several cardiac and vascular factors, but it is the role of large conduit arteries, mainly the aorta, to minimize pulsatility. However, whether PP contributes to the development of atherosclerosis or if atherosclerosis leads to pulse pressure widening remains unclear.

PURPOSE: To examine whether exposure to large PP in adolescence predicts carotid artery intima-media thickness (cIMT) and carotid stiffness indices in adulthood.

METHODS: We assessed 79 participants from the European Youth Heart Study (Portugal). Baseline assessment was conducted in 1999/2000 in adolescents aged 15-16 years old and these participants were followed-up into young adulthood (29-31 years old). Baseline and follow-up assessment included measures of systolic (SBP) and diastolic (DBP) blood pressure and PP was calculated. In young adulthood cIMT and arterial stiffness were assessed using a high-resolution ultrasound scanner. PP and vascular health markers were dichotomized into normal and low risk according to the highest risk tertile. Linear and logistic regression analyses were performed.

RESULTS: Adolescence PP was associated with cIMT and stiffness (distensibility coefficient and stiffness indices α and β). Changes in PP from adolescence to adulthood were negatively related to the compliance coefficient in adulthood. Logistic regression analysis demonstrated that regardless of adulthood PP, adolescents with increased PP were at risk for presenting increased cIMT in adulthood. Additionally we verified that increased PP in adulthood, but not adolescence, was related to increased stiffness (stiffness indices α and β).

CONCLUSIONS: High PP in adolescence may induce changes that contribute to increased cIMT and central stiffness. Whereas cIMT appeared to be influenced by PP during adolescence, arterial stiffness depended primarily on current PP. Furthermore, changes in PP were associated with compliance of the carotid artery.

KEYWORDS

Atherosclerosis; carotid intima media thickness; carotid stiffness indices; epidemiology

INTRODUCTION

For many years systolic and diastolic blood pressure were the exclusive mechanical factors predicting cardiovascular risk in populations of normotensive and hypertensive individuals. However, if hypertension acts as a mechanical factor with deleterious consequences on the arterial wall, the totality of the blood pressure curve should be considered to evaluate the risk. Other hemodynamic indices with particular relevance for cardiac complications and that originate from pulsatile pressure have scarcely been taken into account, namely brachial pulse pressure (PP) and aortic pulse wave velocity. A current approach consists of considering the blood pressure curve as the summation of a steady component, mean arterial pressure (MAP), and a pulsatile component, PP. MAP, the product of cardiac output multiplied by total peripheral resistance, is the pressure for the steady flow of blood and oxygen to peripheral tissues and organs. The pulsatile component, PP, is the consequence of intermittent ventricular ejection from the heart. PP is influenced by several cardiac and vascular factors, but it is the role of large conduit arteries, mainly the aorta, to minimize pulsatility.

Increased PP is an independent predictor of myocardial infarction, congestive heart failure and cardiovascular death, even in hypertensive patients undergoing successful antihypertensive drug therapy. Increased aortic pulse wave velocity and increased carotid incremental elastic modulus are also both independent predictors of cardiovascular mortality, mainly in patients with end-stage renal disease and, to a lesser extent, in individuals with essential hypertension [466].

Although the clinical complications of coronary heart disease mainly occur in middle age or later in life, atherosclerosis has its roots in childhood. Cardiovascular risk factors identified in childhood and adolescence have consistently been shown as predictors of increased intima-media thickness (cIMT) [4] and carotid artery elasticity [447] in adulthood. Systolic blood pressure (SBP) in childhood is associated with arterial structure and stiffness in adulthood [4, 89, 222, 446, 447]. However, studies on the contribution of PP to the development of atherosclerosis have only examined its prediction ability on cIMT [448]. Therefore, to gain more insight on childhood determinants of adult vascular health, we measured several carotid stiffness indices - carotid compliance coefficient (CC), distensibility coefficient (DC) and stiffness index α and β in 79 young adults aged 29-31 years of age. These individuals were participants of the prospective Cardiovascular Risk in European Youth Heart Study for whom risk factor data were available since their adolescence.

METHODS

PARTICIPANTS

The European Youth Heart Study is an ongoing multi-national study [467] in which several countries participated: Denmark, Estonia, Portugal and Norway.

A cross-sectional survey was conducted in 1999/2000 for 592 children (50.5% girls) aged 15-year-old from Madeira Island, Portugal. In this first evaluation, youths were measured in the Department of Physical Education and Sport of Madeira University. In 2013 we have started reexamining these individuals, now aged 31 years.

ANTHROPOMETRY

Weight and height were measured while the participants were wearing light clothing and no shoes, using standard techniques. Height was measured to the nearest 0.5 cm using a Seca stadiometer (Medical Scales and Measuring Systems Seca ltd, Birmingham, UK) and weight was measured to the nearest 0.1 kg with a calibrated Seca beam balance scale (Medical Scales and Measuring Systems Seca ltd, Birmingham, UK). Body mass index

(BMI) was calculated as the weight in kilogrammes divided by the square of height in meters (kg/m^2).

BRACHIAL BLOOD PRESSURE

Resting SBP and diastolic blood pressure (DBP) were measured after 5 min of sitting rest, with a Dinamap XL Vital signs monitor, (Johnson & Johnson Medical Inc., Arlington, TX, USA). Five measurements were taken at 2 min intervals and the mean of the final three measurements was used in all of the analyses. Mean arterial pressure (MAP) was calculated as follows: MAP = (1/3 PP + DBP). PP was calculated as follows: PP = SBP-DBP.

CAROTID ARTERY STIFFNESS INDICES AND BLOOD PRESSURE BY VASCULAR ULTRASOUND

The carotid artery stiffness measurement was conducted with the patient in the supine position after at least a 15 min resting period before. We used a ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy) with Quality Arterial Stiffness technology, based on radio frequency signal in a common carotid artery segment ~1 cm before the bifurcation. This software used a complex algorithm that could process all data coming from the arterial wall as radiofrequency signals, automatically measuring the modification of the arterial diameter between the systolic and diastolic phases. During imaging of the common carotid artery, the examiner obtained real-time feedback on measurement to optimize the probe position to have best scan plane with respect to the distension and diameter. Theoretically, carotid diameter waveforms were assessed by means of ultrasound and converted to carotid pressure waveforms using an empirically derived exponential relationship between pressure and arterial cross-section. The derived right carotid pressure waveform was calibrated to right brachial end diastolic and mean arterial pressure by iteratively changing the wall rigidity coefficient. This allows the calculation of carotid stiffness indices: distensibility coefficient (1/KPa), compliance coefficient (mm2/kPa), stiffness index α and β. DC is the fractional change in cross-sectional area relative to the change in arterial pressure. DC was calculated as: $DC = \frac{\Delta \mathrm{A}}{\mathrm{A} \cdot \Delta PP} = \frac{2 \cdot \mathrm{D} \cdot \Delta \mathrm{D} + \Delta D^2}{D^2 \cdot \Delta PP}$ where A: diastolic area; ΔA: change of area in systole. Compliance

Coefficient (CC) is calculated as: $CC = \frac{\Delta A}{\Delta PP} = \frac{\pi \cdot (2 \cdot D \cdot \Delta D + \Delta D^2)}{4 \cdot \Delta PP}$; Stiffness index α was expressed as: $\alpha = \frac{A \cdot \ln\left(\frac{Ps}{P_d}\right)}{\Delta A} = \frac{D^2 \cdot \ln\left(\frac{Ps}{P_d}\right)}{2 \cdot D \cdot \Delta D + \Delta D^2}$ and stiffness index β was expressed as: $\beta = \frac{D \cdot \ln\left(\frac{Ps}{P_d}\right)}{\Delta D}$, where Ps and Pd are carotid systolic and diastolic pressure respectively.

The coefficients of variation for repeated measurements in our laboratory for DC, CC, stiffness index α and β, carotid SBP and DBP are: 0%, 2.87%, 3.70%, 3.18%, 2.15% and 0%, respectively.

STATISTICAL ANALYSES

Descriptive values were expressed as mean ± standard error of the mean. Baseline group characteristics were compared with paired-sample t-tests. Multivariate and logistical regression models (set by tertiles) adjusted for sex and BMI changes were used to examine the associations between youth PP and respective life-course trajectories with carotid stiffness and wall thickness. Regression values were expressed as β ± 95%CI.

Repeated measures analysis of variance was used to compare PP and respective life-course trajectories between groups with increasing gradients of carotid stiffness and wall thickness. To analyze the extent to which the concomitant life-course of other risk factors explained any of the differences found between groups, analyses were further adjusted for potential mediators (MAP and BMI).

Statistical significance level was set at $p<0.05$ for all tests. The statistical analyses were computed and analyzed by a certified researcher using the SPSS Statistics 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The characteristics of the study population are shown in TABLE 20. BMI, SBP, DBP and MAP increased from adolescence to young adulthood ($p<0.05$). The prevalence of overweight and obesity in adolescence was 17.5% and increased to 45% in young adulthood ($p<0.05$) [139, 468]. SBP was increased in 5% of young adults [469] but not in

adolescence [112]. The carotid blood pressure and carotid stiffness indices in young adults are in line with the reference values reported in literature [391, 393]

Results from linear regression analysis between PP and cIMT and arterial stiffness indices are presented in TABLE 21. Significant associations were found between adolescence PP and cIMT and arterial stiffness indices (DC, stiffness indices α and β).

Subjects with stiffer arteries (T1 of the carotid DC) had on average and throughout the whole longitudinal period greater levels of PP (6.91 ± 1.87 mmHg), than those with less stiff arteries (T2 + T3; FIGURE 19). Importantly, this difference was constant over time (p=0.808), being already present during adolescence (p=0.06). Adjustment for current BMI and MAP and changes in BMI and MAP did not attenuate the differences in PP (5.40±1.93; 6.90± 1.88; 6.40± 1.92; 6.80±1.87 mm Hg, respectively), which remained significant (p<0.05).

TABLE 20: PARTICIPANTS' CHARACTERISTICS AT BASELINE (ADOLESCENCE) AND FOLLOW-UP (ADULTHOOD).

		Adolescence			Adulthood		
Age	(years)	15.65	±	0.04	30.05	±	0.06*
BMI	(kg/m^2)	21.29	±	0.30	24.84	±	0.43*
SBP	(mmHg)	105.64	±	1.14	111.63	±	1.08*
DBP	(mmHg)	59.56	±	0.75	66.13	±	0.89*
MAP	(mmHg)	74.92	±	0.77	81.30	±	0.84*
Brachial PP	(mmHg)	46.07	±	1.00	45.51	±	0.96
Carotid PP	(mmHg)				35.97	±	0.89
cIMT	(mm)				0.54	±	0.01
DC	(1/Kpa)				0.039	±	0.001
CC	(mm2/kPa)				1.38	±	0.04
Stiffness Index α					3.03	±	0.08
Stiffness Index β					6.30	±	0.17

Results are mean ± SEM; *Significant differences p<0.05

Subjects with thicker arteries (T3 of the cIMT; FIGURE 20) had greater levels of PP during adolescence (5.96 ± 2.06 mmHg; p=0.005), but not in adulthood (p=0.212), independent of past levels of BMI and MAP, and changes in BMI and MAP (5.02±2.03; 5.64± 2.01; 5.79± 2.074; 5.69±2.04 mmHg, respectively), which remained significant (p<0.05).

TABLE 21: MULTIVARIATE ASSOCIATION BETWEEN PULSE PRESSURE (ADOLESCENCE AND CHANGES TO ADULTHOOD) AND SUBCLINICAL MARKERS OF ATHEROSCLEROSIS AT ADULTHOOD.

	cIMT	DC	CC	Stiffness index α	Stiffness index β
PP at adolescence	0.273 (0.039, 0.507) 0.023	-0.312 (-0.533, -0.091) 0.006	-0.129 (-0.369, 0.111) 0.287	0.326 (0.097, 0.556) 0.006	0.334 (0.105, 0.562) 0.005
Changes in PP	-0.196 (-0.426, 0.035) 0.095	-0.155 (-0.378, 0.067) 0.169	-0.343 (-0.564, -0.122) 0.003	0.141 (-0.091, 0.373) 0.228	0.153 (-0.079, 0.384) 0.192

Results are presented as β standardized coefficients, 95% CI and p value

FIGURE 21 illustrates the odds-ratio for increased risk of subclinical atherosclerosis by PP tracking category. Logistic regression analysis demonstrated that participants in the high PP category in adolescence were at risk for increased cIMT in adulthood, even if they change into normal PP values in adulthood.

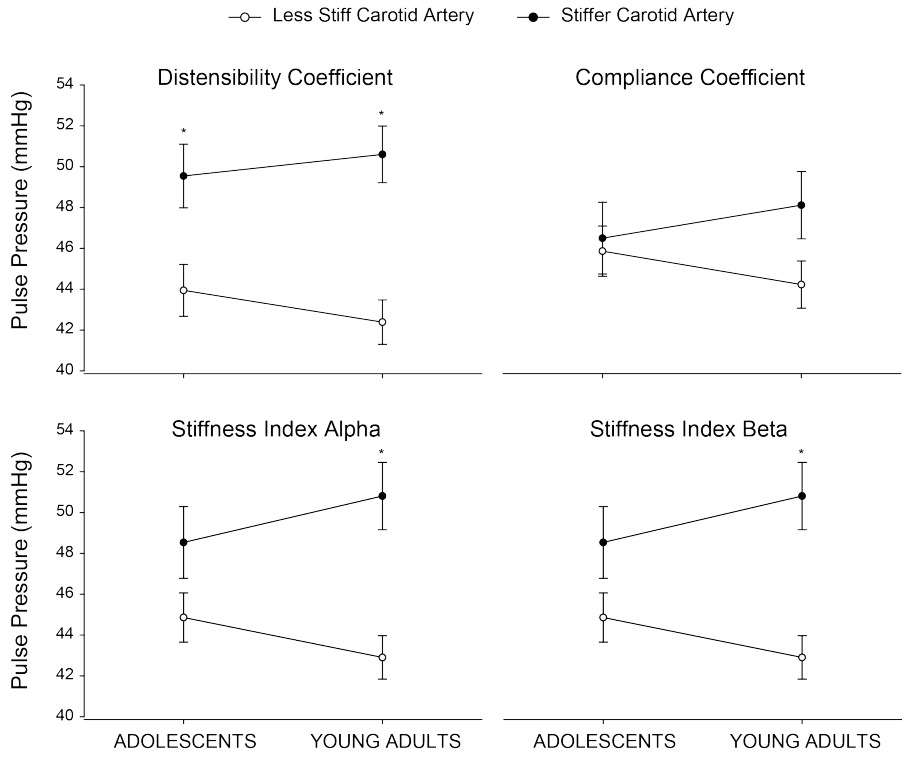

FIGURE 19: COMPARISON OF THE LIFE-COURSE TRAJECTORIES OF PP BETWEEN SUBJECTS WITH STIFFER VS LESS STIFF CAROTID ARTERIES AT YOUNG ADULTHOOD.

All data are adjusted for sex.
*P0.05 for comparisons between subjects with stiffer vs less stiff carotid arteries.

For stiffness variables index α and β we observed that, regardless of adolescence PP, participants with higher PP in adulthood were at risk for increased stiffness.

DISCUSSION

We investigated the trajectories of PP from adolescence to adulthood and examined its potential role as a determinant of carotid stiffness and cIMT. We showed that 1) as compared with individuals with less stiff arteries at age 30 years, those with stiffer arteries were characterized from ages 15 to 30 years by greater levels of PP, independent of BMI and MAP; 2) adolescents with increased PP were at risk for increased cIMT in young adulthood regardless of current distending pressure; 3) increased PP in young adulthood, but not adolescence, was related to increased stiffness.

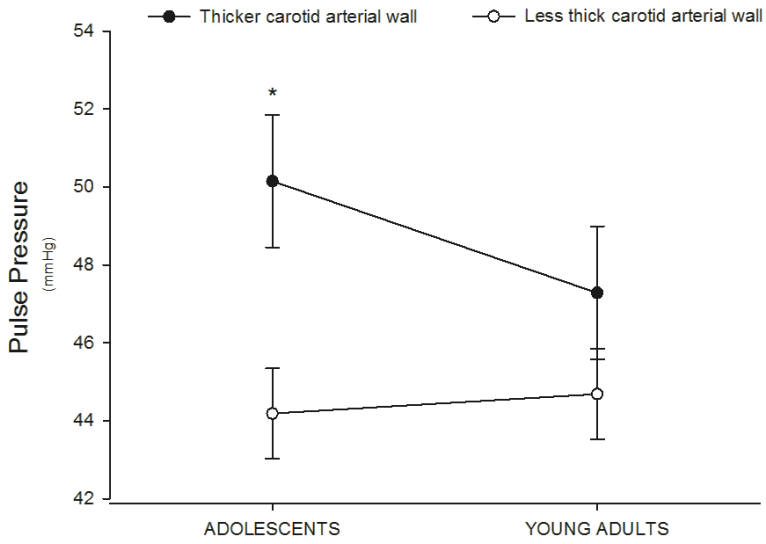

FIGURE 20: COMPARISON OF THE LIFE-COURSE TRAJECTORIES OF PP BETWEEN SUBJECTS WITH THICKER VS LESS THICK CAROTID ARTERIAL WALL AT YOUNG ADULTHOOD.

All data are adjusted for sex.
*P0.05 for comparisons between subjects with stiffer vs less stiff carotid arteries.

High pressure pulsation may directly influence atherosclerosis by a number of mechanisms [470], including endothelial dysfunction [471], enhance adhesion of monocytes to endothelial cells [472, 473] and modulate gene expression in smooth muscle cells and monocytes/macrophages [470, 474]. However, the limited number of longitudinal studies that has addressed the association of PP in adolescence and subclinical

atherosclerosis in adults has only used cIMT as a surrogate vascular marker. In relatively aged subjects in the Epidemiology of Vascular Aging study, PP was associated with the 4-year change in cIMT and cIMT was associated with the change over time in PP [475]. These associations were independent of MAP and other traditional cardiovascular risk factors and were observed both in hypertensive and non-hypertensive subjects as well as in antihypertensive-treated and non-treated subjects. The consistent lack of association between MAP and cIMT, after adjustment for PP, both in Zureik et al. [475] cross-sectional and longitudinal analyses, is noteworthy, suggesting that at least in the elderly, the relationship of MAP with cIMT is far less important than that of PP. In The Young Finns Study, exposure to large PP in adolescence was independently associated with increased cIMT in adulthood [448]. We extend these findings by showing that lowering blood pressure at adulthood may not be enough to throw back vascular remodeling, or it may take longer to reduce cIMT. In addition, this longitudinal approach enabled us to pinpoint adolescence as the period early in life when increases in PP may be linked to greater cIMT later in life.

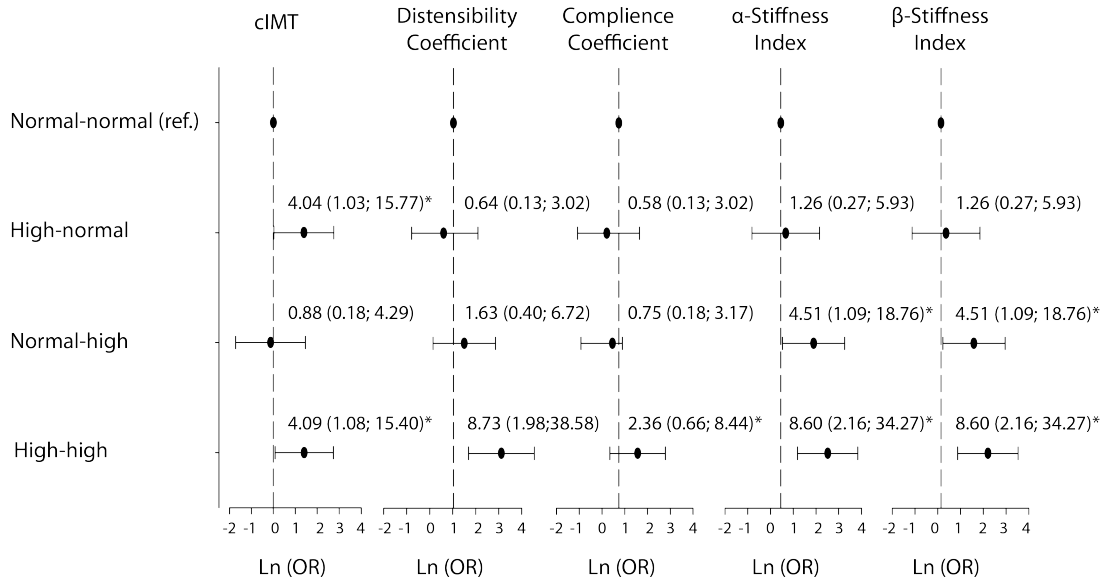

FIGURE 21: LOGISTIC REGRESSION ANALYSIS BETWEEN PP RISK CHANGES INTO ADULTHOOD WITH RISK FOR SUBCLINICAL ATHEROSCLEROSIS.

Results are presented as odds-ratio and 95% CI

Recent studies did not use PP when examining whether the blood pressure trajectories from adolescence to young adulthood determined levels of arterial stiffness in young adults [449]. Subjects with stiffer arteries (as given by DC, CC or Young's elastic modulus) had on average and throughout the whole longitudinal period greater levels of MAP, SBP, and DBP, respectively, than those with less stiff arteries. Importantly, these differences were not constant over time, and although being already present during adolescence were further amplified from this age onward and more strongly so in subjects with stiffer arteries, resulting in differences between the 2 groups that were 2.5-fold greater at age 36 years [449]. In agreement with previous observations [446, 447, 449, 476], we showed that young adults with stiffer arteries (T1 for carotid DC) had greater levels of blood pressure early in life. In addition, we extended previous findings by showing that a PP reduction in young adulthood may induce significant benefits in arterial stiffness (DC, Stiffness Index α and β) as opposed to what we found for cIMT. This suggests that alterations in vasomotor tone may account for changes in arterial wall structure, in line with the idea that changes in arterial stiffness precede those of echographic measures [38]. In the present study, subjects with less stiff carotid arteries (T3 of the carotid DC) were those who, between adolescence and young adulthood, had consistently lower PP (barely exceeding the mean values of 42 mmHg, respectively, at age 30 years). In this respect, Ferreira et al. [449] suggest that subjects with less stiff carotid arteries were those who, between adolescence and young adulthood, had relatively lesser increases in SBP and DBP (barely exceeding the mean values of 130 and 85 mmHg, respectively, at age 36 years). These values could be used as references for a healthy profile within the age periods as examined herein.

Increased arterial stiffness is primarily determined by the properties of the extracellular matrix (elastin, collagen) and vascular smooth muscular cell function [477-479]. These properties are strongly affected by lifelong blood pressure [480, 481]. Young adult hypertensive subjects have a "downstream" increase in resistance at the level of the arterioles, causing an "upstream" increase in transmural pressure at the level of the central elastic arteries, causing weight-bearing elastic lamellae of the large arteries to stretch and become stiffer [480]. This form of hypertension in young adults typically presents with elevated DBP in the subtypes of systolic–diastolic or isolated diastolic hypertension in contrast with the isolated systolic hypertension of the elderly, associated with increased SBP and decreased DBP, typically presented with widened PP. As large arteries dilate,

wall tension and pulsatile stress increase and exacerbate artery wall degeneration, thus initiating a positive feedback whereby increased hypertension leads to further degeneration [480]. To figure out the form of hypertension predominant in this study we ran an additional multiple regression analysis with arterial stiffness indices as dependent variables and, sex, BMI changes and blood pressure as independent variables (data not shown). We found that our data is consistent with the subtypes of elevated SBP and DBP (when using current blood pressure values) or isolated elevated DBP (when using changes in blood pressure) that typically characterize young adults.

LIMITATIONS

Our study has limitations. The tertiles set by the authors for cIMT, carotid stiffness indices and PP may not predict risk at all. A revised version of this manuscript should take into account the reference values for clinic pulse pressure in a non-selected population by Asmar et al. [482] and the 95th percentile as abnormal with 85th - 95th percentile as "at risk" for subclinical atherosclerosis.

Increased cIMT can be seen in children with risk factors. Therefore, it is possible that the early increase in PP in the adolescents is a result rather than a cause of increased cIMT. However, our results provide further evidence for the hypothesis that the pulsatile component of blood pressure has a role in the development of increased cIMT and stiffness [448, 449].

Our findings were confined to subjects in whom complete data for arterial properties were obtained during the follow-up examination from 2013-2015. We have not examined if levels of BP, total and central fatness, blood lipids, cardiorespiratory fitness, and HR in these subjects differed, at the earlier time point, from those subjects who dropped-out. This could indicate that selection bias may threat the validity of our findings.

Although the use of local PP estimated by calibration of the distension waveforms instead of brachial PP for the calculations of the carotid stiffness indices constitutes strength to our study, this method still may not optimally reflect the level of PP at the level of the carotid artery.

Given its observational design, this study cannot prove causality. Because carotid stiffness levels were measured at age 30 years only, we cannot rule out the possibility of reverse causality. However, we deemed this less likely because the young subjects were, throughout the whole longitudinal period, unaware of and asymptomatic with regard to their stiffness levels.

CONCLUSION

Exposure to wide PP in adolescence may induce changes that contribute to increased cIMT and central stiffness. Whereas cIMT appeared to be influenced by PP during adolescence, arterial stiffness depended primarily on current PP.

CHAPTER 4

WHERE ARE WE AND WHERE ARE WE GOING?

1. WHERE ARE WE?

The precursors of premature CVD begin in youth. Treatment of CVD in symptomatic adults is at best palliative in most cases, emphasizing the need for diagnosis and prevention at a much earlier stage of this disease [5].

Given the impracticality of and ethical issues related to lengthy placebo-controlled trials, the use of surrogate vascular markers is an excellent approach. Surrogate vascular markers can be used to follow the evolution of CVD, the cumulative effects of risk factors, the effects of any risk factor modification on arterial health and the efficacy of nutritional, pharmaceutical therapy and exercise in promoting adaptations in arterial wall properties. Using these tools, the intensity and timing of therapeutic interventions could be adjusted based not only upon conventional lipid levels, glucose levels, blood pressure, weight, etc., but rather on markers of subclinical disease.

PRINCIPAL FINDINGS

Considering the effects of aging on arterial stiffness and left ventricular relaxation patterns, obesity-related advancements in vascular age and cardiac dysfunction during childhood will accelerate the progression of CVD. What is most apparent from the collective findings in this dissertation is that risk factors in childhood not only increases cardiovascular risk in adulthood, but is also associated with cardiovascular damage during childhood.

We have found that in a pediatric population without specific exclusion criteria other than been apparently healthy, differences exist in body composition variables among cIMT tertiles and that even moderate degrees of fatness, in particular regional fatness, influence arterial structural and are related to cardiovascular risk. The combination of total body fat assessment and central pattern of fat accumulation measures does not improve the prediction of increased cIMT in children. Thus, special attention should be urged to those children who exhibit an increased central body fat phenotype, a stronger predictor of arterial structure even when compared to SED, MVPA and CRF. All the same, our results show that children failing to meet CRF standards have significantly increased cIMT compared with those who meet them. Thus, health-related fitness cut-offs for children and adolescents can as well be used as a risk stratification tool to identify target populations for

health promotion policies and disease prevention in public health and clinical settings. Although criterion-based measurement of aerobic fitness is difficult and expensive to assess, estimation from aerobic performance is easy and reliability is sufficient for this purpose. Prevention programs should also focus on improving physical fitness during childhood. In fact, we have shown that muscular strength is inversely associated with cIMT independently of PP, central fatness and CRF. Children with low muscular strength have the highest cIMT values, WC and SBP coupled with the low CRF, apparently setting the stage for increased risk of cardiovascular complications in adulthood.

We are not advocating physical training regimes or adult fitness training criteria for children. Rather we believe that, in addition to physical activity, physical fitness should be viewed as an important cardiovascular attribute and that improving physical fitness levels should be tested as a specific intervention strategy against early signs of vascular pathology. In the end, our studies suggest that low physical fitness may be a pediatric cardiovascular risk factor independent of (or perhaps interacting with) low levels of physical activity [483] and overweight/obesity.

Many of the potentially favorable changes in atherosclerotic cardiovascular disease risk factors previously considered to require long-term exercise training are now known to have both an acute and chronic exercise component [484]. Isolated exercise sessions elicit acute, transient cardiovascular, and metabolic responses. Frequent repetition of these isolated sessions produces more permanent adaptations, referred to as the exercise training response. These risk factors include blood lipids, blood pressure, and serum glucose, but many of the non-structural changes on arterial properties that occur with exercise training are also affected by recent exertion. We have shown that an acute bout of maximal exercise can alter arterial stiffness and hemodynamics in vasculatures perfusing the brain and within the active muscle beds. The changes in stiffness may be related to the hemodynamic response and arterial stiffness and hemodynamic response to metabolic demands during exercise in children simply reflect their smaller body size and may not indicate a particular physiologic difference compared to adults.

Certainly there is a high interdependence of arterial wall properties and arterial pressure in children and adults [43, 90, 409, 417]. However, there is some controversy on the competing effects of age, and of blood pressure change accompanying age, on central

artery dilation. Cross-sectional studies cannot be expected to resolve this issue [409]. However, we have shown longitudinally that exposure to wide PP in adolescence may induce changes that contribute to increased cIMT and central stiffness in adulthood. Whereas cIMT appeared to be influenced by PP during adolescence, arterial stiffness depended primarily on current PP. Furthermore, changes in PP were associated with compliance of the carotid artery. It is known that the acute and chronic depressor effects of dynamic exercise are a low-threshold phenomenon with hypotensive responses noted at an exercise intensity of 40% of maximum oxygen consumption [485, 486] and after just three sessions of aerobic activity in training studies [486]. The depressor influence of exercise quickly subsides with blood pressure increasing to pre-exercise levels after 1–2 weeks of detraining [487].

The call for action is set: not only there is strong evidence that preclinical CVD in adulthood is preceded by differences in modifiable risk factors that are detectable in youth, as data indicates that health-enhancing changes in physical fitness during the transition from youth to adulthood are important in modifying an individual's trajectory from high risk in youth to low risk in adulthood [456].

CLINICAL AND RESEARCH CONSIDERATIONS

The research to date illustrates that risk factors during childhood and adolescence is associated with various measurable alterations in vascular structural and elastic properties. Interpretation of research findings is complex, given the increased variation in the type and number of cardiovascular risk factors present in children. Our understanding of the cumulative effects of multiple risk factors, the weighted importance of the risk factor, and the effect of the length of exposure to the risk factors is limited.

In the clinical setting, the measures discussed in this dissertation are not routinely performed. Thus, the extent of cardiovascular changes related to particular phenotypes of body composition and hemodynamic profile are likely under-recognized and therefore undermanaged. A standardized clinical approach to the cardiovascular evaluation of children with multiple risk factors is required. Importantly, this approach should include early detection and evaluation of subclinical cardiac dysfunction, given the potential for reversibility. However, incorporating these assessments into clinical practice is challenging

when specialized equipment and/or personnel may be required. Furthermore, there is a lack of adequate normative data with which to compare the individual patient, and acquisition of these data is paramount to establishing clinical guidelines.

For the clinician, monitoring cardiovascular biomarkers may be a more attractive option for individual patient care as lipid screening is already part of routine assessment and monitoring in children with obesity for example. Biomarkers may indicate the progression of disease but causality cannot be assumed. Despite associations between biomarkers and non-invasive measures of early atherosclerosis in children continue to emerge and several biomarkers appear to be promising, a review of CVD biomarkers in children concluded that there are no explicit data to recommend any biomarkers as a routine clinical marker of CVD in children. More work is needed to validate biomarkers and to improve understanding of their role in CVD risk prediction in the pediatric population [488].

ARE ALL RISK FACTORS IMPORTANT?

All the major established risk factors accelerate the progression of atherosclerosis in the teenage and young adult years, and their effects are cumulative. Although risk factor effects vary in magnitude and in the arterial segments affected, no established risk factor can be safely ignored [432].

WHEN TO START?

A healthy lifestyle is a keystone to reduce the impact of inherited CVD risk factors. But at what age should children acquire health-promoting habits or interventional therapy be initiated to modify risk factors and to what extent must they be reduced to favorably impact on longitudinal health? These important questions must be answered with studies aimed at tracking healthy habits, determining the effects of risk factors both singularly and in combination as well as the efficacy of any risk factor-modifying intervention on markers of actual disease regression or stabilization.

Limited information is available on normal values by age, race/ethnicity, sex and how they differ by body/arterial size and with normal growth. Most important, the effect reduced arterial compliance in childhood has on arterial compliance and risk of CVD in adulthood has not been elucidated to date. We also do not know if reducing the risk factors in

childhood, leading to improvement in arterial structure and compliance, will affect the risk for cardiovascular events in adulthood.

It would be most effective to begin to control risk factors early in life, but findings from the Pathobiological Determinants of Atherosclerosis in Youth show that risk factors are associated with all stages of atherosclerosis indicating that risk factor control is likely to be beneficial regardless of the stage of disease when control is implemented [432].

More studies will be needed to tease out the age at which differences become apparent and whether these associations extend to other adult preclinical markers. Moreover, the impact of changing from metabolically healthy overweight/ obese youth to a metabolically unhealthy overweight/obese adult (or vice-versa) and the factors associated with these changes need to be determined.

CAN THE PREDICTION OF ATHEROSCLEROSIS BE IMPROVED IN YOUTH?

Several risk scores can identify a young person at high likelihood of having advanced atherosclerosis [464, 489, 490]. Non-invasive techniques applied to high-risk individuals can separate those with advanced lesions from those without, to guide individual interventions. Longitudinal studies using non-invasive measurements of atherosclerosis will permit researchers to refine these risk scores.

ARE CLINICAL TRIALS NECESSARY BEFORE RISK FACTOR CONTROL IS IMPLEMENTED IN YOUTH?

The evidence supporting the usefulness of risk factor control in young people is based primarily on observational studies and inferences from studies of adults. Except for statin treatment of young individuals with familial hypercholesterolemia [491], no controlled clinical trial has been conducted. Any trial that requires random assignment to lifestyle modification (healthy diet with caloric intake balanced with physical activity, avoidance of smoking) and evaluation for 15 or more years of follow-up does not appear to be feasible and perhaps is even unethical [432]. The evidence demonstrating absence of harm and the benefits of preventing the development of CVD risk factors is sufficient to justify aggressive promotion of risk factor control in youth now. Evaluation of the benefits of risk factor control in young persons in shorter trials can use non-invasive methods, such as

those used in assessing the effects of lowering blood lipids in children [491], and measurement of functional markers associated with atherosclerosis [447, 492].

WHAT ARE THE MECHANISMS?

Little is known regarding the mechanisms that cause the alterations in arterial structure and stiffness in youth with cardiovascular risk factors. Furthermore, the most effective way to intervene and improve arterial structure and compliance in youth with cardiovascular risk factors remains speculative. In particular, the most appropriate exercise prescription, type of diet, supplementation regime, or combination thereof have not been fully described.

Much work remains to optimize recommendations for exercise activities in children, both healthy and overweight/obese. The exercise research community needs to define the type, frequency, intensity, and duration of exercise to maximize benefit. It is fundamental to understand which age groups benefit the most from intervention and which disease populations require which type of diet and exercise intervention.

WHICH FACTORS NEED TO BE TAKEN INTO ACCOUNT IN THE APPLICATION OF ARTERIAL STIFFNESS IN THE ACUTE EXERCISE MODEL?

Further research is warranted to validate the use of arterial stiffness with acute exercise in children. In particular, future investigations should 1) examine the arterial stiffness diurnal rhythm and determine time periods of stable arterial stiffness in children; 2) identify the optimal time to measure post-exercise arterial stiffness; 3) compare the effect of different modalities of acute exercise on arterial stiffness to determine whether exercise produces a localized or systemic effect; 4) determine the impact of exercise-induced sympathetic activity modulation on the mechanism of the arterial stiffness response. Some of these questions could be approached by incorporating flow-mediated dilation measurement before and after exercise. In addition, studies are needed to determine whether changes in arterial stiffness and flow-mediated dilation induced by acute exercise are accompanied with changes in other accepted markers of endothelial function such as circulating adhesion molecules (ICAM-1, VCAM-1, E-selectin) and Von Willebrand factor [352].

This dissertation has given us important motivation to progress along this challenging trail.

2. WHERE ARE WE GOING?

The oft stated refrain of those involved in children's healthcare is true: "Children are not little adults." Thus, the implementation of nutritional, pharmaceutical therapy and exercise in youth should be based on data in this unique population, rather than extrapolated from adult data. More intervention studies in youth are desperately needed to guide the therapeutic management of risk factors as well as providing a better understanding of the early natural history of this disease. At no time has this need been more acute as it is currently with the rising prevalence of childhood obesity and sedentary behaviors. Moreover, in an era of escalating medical costs there should be strong evidence that risk factor screening and implementation of any disease-modifying intervention is targeted and efficacious in the pediatric population [5]. Ultimately, the future health of our youth will depend on carefully balancing the benefits of early treatment with the long-term risk of increased morbidity and mortality due to CVD.

We will now focus on imaging and non-imaging modalities that are promising tools to expand our understanding of the cardiovascular risk imposed on youths. This selection was based on a profound and balanced reflection on the human know-how and equipment acquisition potential existent at present in the Laboratory of Health and Exercise of the Faculty of Human Kinetics – University of Lisbon.

CARDIAC AND ARTERIAL STRUCTURE AND FUNCTION

The explosion of advanced non-invasive imaging modalities has allowed further understanding of the early involvement of the heart and vasculature in CVD with future potential clinical application. These modalities used for determining vascular function include plethysmography of the brachial artery to determine flow-mediated dilation and brachial distensibility.

BRACHIAL ARTERY ULTRASOUND TO DETERMINE FLOW-MEDIATED DILATION

Flow-mediated dilation studies such as brachial reactivity is performed by exposing local vasculature to ischemia and monitoring its nitric oxide-mediated vasodilation as reflected

by increased flow. Studies show impaired endothelium-dependent flow-mediated dilation, indicating vascular dysfunction in children and adolescents with positive family history for CVD, familial hyperlipidemia, and obesity [79].

ECHOCARDIOGRAM

Studies of children have reported that left atrial and left ventricular dimensions [493] are significantly greater in children with obesity compared with children with a healthy BMI. Greater epicardial fat has also been reported in children with obesity compared with sex-matched children with a healthy BMI, and is positively associated with left ventricular mass [494].

Altered cardiac morphology may be a precursor to impaired cardiac function, or alternatively, cardiac dysfunction may elicit changes in chamber morphology. Studies of children and adolescents with obesity have reported altered cardiac mechanics including diastolic dysfunction [495] and systolic dysfunction at rest [496] and during exercise [497].

Even with normal ejection fraction, myocardial contractile abnormalities may still exist, and are typically highlighted with more sensitive measures such as tissue Doppler, strain and strain rate analyses [498]. Indeed, digital tagging by magnetic resonance imaging or speckle tracking imaging with echocardiography, have identified reductions in right and left ventricular strain [493, 499] and strain rate in children with obesity. Impaired contractility as demonstrated by these techniques could be missed with more conventional, limited evaluations, and as such the extent of cardiac dysfunction in childhood obesity may be underestimated.

AUTONOMIC FUNCTION

Impaired cardiac autonomic function is related to all-cause mortality and sudden cardiac events in adults [500]. Indications of reduced vagal or parasympathetic activity of HR variability were also reported [501] with concurrent decrease in sympathetic activity [502] in children. In addition, diminished baroreflex sensitivity has also been reported [503]. Given that the baroreflex is important for regulating blood pressure [504], the assessment of cardiac baroreceptor sensitivity incorporates both afferent and efferent signaling in

cardiac vagal activity and may be more sensitive than HR variability to identify autonomic dysfunction in children [505].

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